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(57) Abstract

A process is provided for modifying growth or architecture of plants by altering the level or the functional level of a cell division controlling protein, preferably a cell–division controlling protein that binds or phosphorylates retinoblasoma–like proteins, more preferably a cyclin, particularly a D–type cyclin within cells of a plant. Also provided are chimeric genes comprising a transcribed DNA region encoding an RNA or a protein, which when expressed either increases or decreases the level or functional level of a cell–division controlling protein, and plant cells and plants expressing such chimeric genes.

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PLANTS WITH MODIFIED GROWTH

This invention relates to the use of cell-division controlling proteins or parts thereof, preferably cell-division controlling proteins that bind retinoblasoma-like proteins, more preferably cyclins, particularly D-type cyclins and genes encoding same, for producing plants with modified phenotypes, particularly plants with modified growth rates or plants comprising parts with modified growth rates and/or modified relative sizes or plants with modified architecture. This invention also relates to plant cells and plants expressing such DNAs.

BACKGROUND TO THE INVENTION

All eukaryotic cells undergo the same sequential series of events when they divide, and the term "cell cycle" reflects the ordered nature and universality of these events. In the eukaryotic cell cycle DNA replication (S) and cell division (M) are normally temporally separated by "gap" phases (G1 and G2) in the sequence G1-S-G2-M. This arrangement allows entry to the critical processes of DNA replication and mitosis to be precisely controlled. Underlying the cytological events of the cell cycle is an ordered series of temporally and spatially organised molecular and cellular processes which define the direction and order of the cycle. Cell cycle progression appears to be regulated in all eukaryotes by major controls operating at the G1-to-S phase and G2-to-M phase boundaries. Passage through these control points requires the activation of cyclin-dependent kinases (CDKs), whose catalytic activity and substrate specificity are determined by specific regulatory subunits known as cyclins and by interactions with other proteins that regulate the phosphorylation state of the complex (reviewed in Atherton-Fessier *et al.*, 1993; Solomon, 1993). In budding and fission yeasts, both the G1-to-S and G2-to-M phase transitions are controlled by a single CDK, encoded by the *cdc2+* gene in *Schizosaccharomyces pombe* and by *CDC28* in *Saccharomyces cerevisiae*. The association of $p34^{cdc2}$ ($p34^{CDC28}$ in budding yeast) with different cyclin partners distinguishes the two control points (reviewed in Nasmyth, 1993). In mammalian cells, a more complex situation prevails, with at least six related but distinct CDKs (encoded by *cdc2/cdk*1, *cdk*2, *cdk*3, *cdk*ain 4, *cdk*5, and *cdk*6) having distinct roles, each in conjunction with one or more cognate cyclin partners (Fang and Newport, 1991; Meyerson *et al.*, 1991, 1992; Xiong *et al.*, 1992b; Tsai *et al.*, 1993a; van den Heuvel and Harlow, 1993; Meyerson and Harlow, 1994). B-type cyclins are the major class involved in the G2-to-M transition and associate with $p34^{cdc2}$ or its direct homologs (reviewed in Nurse, 1990). Cyclin B is one of two cyclins originally described as accumulating in invertebrate eggs during interphase and rapidly destroyed in mitosis (Evans *et al.*, 1983), and it is a component of Xenopus maturation-promoting factor (Murray *et al.*, 1989). Subsequently, cyclin B homologs have been identified from many

eukaryotic species. Cyclin A is also of widespread occurrence in multicellular organisms, and its precise role is unclear, although its peak of abundance suggests a function in S phase (reviewed in Pines, 1993).

The G1-to-S phase transition is best understood in *S. cerevisiae*. Genetic studies define a point late in G1 called START. After passing START, cells are committed to enter S phase and to complete a full additional round of division, which will result in two daughter cells again in G1 phase (Hartwell, 1974; reviewed in Nasmyth, 1993). The products of three *S. cerevisiae* G1 cyclin genes called *CLN1, CLN2,* and *CLN3* are the principal limiting components for passage through START (Richardson *et al.*, 1989; Wittenberg *et al.*, 1990; Tyers *et al.*, 1993). Transcription of *CLN1 and CLN2* is activated in G1, and accumulation of their protein products to a critical threshold level by a positive feedback mechanism leads to activation of the $p34^{CDC28}$ kinase and transition through START (Dirick and Nasmyth, 1991). The G1 cyclins are then degraded as a consequence of PEST motifs in their primary sequence that appear to result in rapid protein turnover (Rogers *et al.*, 1986; Lew *et al.*, 1991; reviewed in Reed, 1991).

The *S. cerevisiae* G1 cyclins are at least partially redundant, because yeast strains in which two of the three G1 cyclin genes are deleted and the third placed under the control of a galactose-regulated promoter show a galactose-dependent growth phenotype. Such strains have been used to identity *Drosophila* and human cDNA clones that rescue this conditional *cln*-deficient phenotype on glucose plates when the single yeast *CLN* gene present is repressed (Koff *et al.*, 1991; Lahue *et al.*, 1991; Léopold and O'Farrell, 1991; Lew *et al.*, 1991; Xiong *et al.*, 1991). Human cDNAs encoding three new classes of cyclins, C, D, and E, were identified by this means. Although these cyclins show only limited homology with the yeast CLN proteins, they have proved important for understanding controls that operate in mammalian cells during G1 and at the restriction point at the G1-to-S phase boundary (Pardee, 1989; Matsushime *et al.*, 1992; Koff *et al.*, 1992, 1993; Ando *et al.*, 1993; Quelle *et al.*, 1993; Tsai *et al.*, 1993b). Cyclin E may act as a rate-limiting component at the G1-to-S phase boundary (Ohtsubo and Roberts, 1993; Wimmel *et al.*, 1994), whereas the dependency of cyclin D levels on serum growth factors (Matsushime *et al.*, 1991; Baldin *et al.*, 1993; Sewing *et al.*, 1993) suggests that cyclins of the D-type may form a link between these signals and cell cycle progression.

An important factor involved in the regulation of cell cycle progression in mammals is the retinoblastoma susceptibility gene encoding the retinoblastoma protein (Rb). Rb binds and inactivates the E2F family of transcription factors, and it is through this ability that Rb exerts most of its potential to restrain cell division in the G1-phase. E2F

transcription factors are known to switch on cyclin E and S-phase genes and the rising levels off cyclin E and/or E2F lead to the onset of replication (Nevins, 1992, Johnson *et al.*, 1993). The ability of Rb to inactivate E2F depends on its phosphorylation state. During most of G1, Rb is in a hypophosphorylated state, but in late G1 phase, phosphorylation of Rb is carried out by cyclin-dependent kinases particularly CDK4 complexed to its essential regulatory subunit, cyclin D (Pines ,1995) and CDK2 complexed to cyclin E (at the G1/S boundary) or cyclin A (in S phase). These multiple phosphorylations of Rb cause it to release E2F, which can then, ultimately promote transcription of the S-phase genes.

Plant cells were used in early studies of cell growth and division to define the discrete phases of the eukaryotic cell cycle (Howard and Pelc, 1953), but there is a paucity of data on molecular cell cycle control in plant systems. Plant cells that cease dividing *in vivo* due to dormancy, or *in vitro* due to nutrient starvation, arrest at principal control points in G1 and G2 (van't Hof and Kovacs, 1972; Gould *et al.*, 1981; reviewed in van't Hof, 1985); this is in general agreement with the controls operating in other eukaryotic systems. Although mature plant cells may be found with either a G1 or a G2 DNA content (Evans and van't Hof, 1974; Gould *et al.*, 1981), the G1 population generally predominates. The G1 control point is found to be more stringent in cultured plant cells subjected to nitrogen starvation; these cells arrest exclusively in G1 phase (Gould *et al.*, 1981). Strong analogies thus exist between the principal control point in G1 of the plant cell cycle, the START control in yeasts, and the restriction point of mammalian cells.

Antibodies or histone HI kinase assays have been used to indicate the presence and localization of active CDC2-related kinases in plant cells (John *et al.*, 1989,1990, 1991; Mineyuki *et al.*, 1991; Chiatante *et al.*, 1993; Colasanti *et al.*, 1993; reviewed in John *et al.*, 1993), and cDNAs encoding functional homologs of CDC2 kinase have been isolated by reduced stringency hybridization or redundant polymerase chain reaction from a number of plant species, including pea (Feiler and Jacobs, 1990), alfalfa (Hirt *et al.*, 1991, 1993), *Arabidopsis* (Ferreira *et al.*, 1991; Hirayama *et al.*, 1991), soybean (Miao *et al.*, 1993), *Antirrhinum* (Fobert *et al.*, 1994), and maize (Colasanti *et al.*, 1991). A number of cDNA sequences encoding plant mitotic cyclins with A- or B-type characteristics or having mixed A- and B-type features have also been isolated from various species, including carrot (Hata *et al.*, 1991), soybean (Hata *et al.*, 1991), *Arabidopsis* (Hemerly *et al.*, 1992; Day and Reddy, 1994), alfalfa (Hirt *et al.*, 1992), *Antirrhinum* (Fobert *et al.*, 1994), and maize (Renaudin *et al.*, 1994).

Soni *et al.* (1995) identified a new family of three related cyclins in *Arabidopsis* by complementation of a yeast strain deficient in G1 cyclins. Individual members of this family showed tissue-specific expression and are conserved in other plant species.

They form a distinctive group of plant cyclins and were named δ-type cyclins to indicate their similarities with mammalian D-type cyclins. The sequence relationships between δ and D cyclins include the N-terminal sequence LxCxE. The leucine is preceded at position -1 or -2 by an amino acid with an acidic side chain (D, E). This motif was originally identified in certain viral oncoproteins and is strongly implicated in binding to the retinoblastoma protein. By analogy to mammalian cyclin D, these plant homologs may mediate growth and phytohormonal signals into the plant cell cycle. In this respect it was shown that, on restimulation of suspension-cultured cells, cyclin δ3 was rapidly induced by the plant growth regulator cytokinin and cyclin δ2 was induced by carbon source. Renaudin *et al.* (1996) defined the groups and nomenclature of plant cyclins and δ-cyclins are now called CycD cyclins.

Dahl *et al.* (1995) identified in alfalfa a cyclin (cycMs4) related to δ3 in alfalfa.

Recently, Rb-like proteins were identified in plant. Both Xie *et al.* (1996) and Grafi *et al.* (1996) describe the isolation and preliminary characterization of an Rb homologue from maize.

Doerner *et al.* (1996) describe the ectopic expression of a B-type cyclin (*cyc*1At from *Arabidopsis*) under control of a promoter from the *cdc*2a gene, in *Arabidopsis*. The "*cdc*2a" transgenic plants expressing the transgene strongly had a markedly increased root growth rate. Moreover, growth and development of lateral roots was accelerated following induction with indoleacetic acid in the transgenic plants relative to the control plants.

Hemerly *et al.* (1995) describe transgenic tobacco and Arabidopsis plants expressing wild type or dominant mutations of a kinase operating at mitosis (CDC2a). Plants constitutively overproducing the wild-type CDC2a or a mutant form predicted to accelerate the cell cycle did not exhibit a significantly altered development. A mutant CDC2a, expected to arrest the cell cycle, abolished cell division when expressed in *Arabidopsis*. Some tobacco plants constitutively producing the latter mutant kinase, were recovered. These plants contained considerably fewer but larger cells.

PCT patent publication "WO" 92/09685 describes a method for controlling plant cell growth comprising modulating the level of a cell cycle protein in a plant for a time and under conditions sufficient to control cell division. The preferred protein, identified in the examples, is a $p34^{cdc2}$ kinase or the like operating at mitosis.

WO93/12239 describes plants with altered stature and other phenotypic effects, particularly precocious flowering and increased numbers of flowers by transformation of

the plant genome with a *cdc*25 gene from a yeast such as *Schizosaccharomyces pombe*.

WO97/47647 relates to the isolation and characterization of a plant DNA sequence coding for a retinoblastoma protein, the use thereof for the control of the growth in plant cells, plants and/or plant viruses as well as the use of vectors, plants, or animals or animal cells modified through manipulation of the control route based on the retinoblastoma protein of plants.

US Patent 5,514,571 discloses the use of cyclin D1 as a negative regulator of mammalian cell proliferation. Overexpression of cyclin D1 blocks mammalian cell growth, while blocking cyclin D1 expression promotes cell proliferation.

SUMMARY OF THE INVENTION

The invention provides a process to obtain a plant with altered growth characteristics or altered architecture, particularly plants with reduced or increased growth rate, plants which require less time to flower or plants with an increased number of flowers per plant, or plant with an increased size of an organ comprising the step of altering the level or the functional level of a cell-division controlling protein, capable of binding and/or phosphorylating an Rb-like protein, preferably a cell-division controlling protein comprising an LxCxE binding motif or related motif, preferably in the N-terminal part of the protein, particularly a D-type cyclin, within the cells of a plant.

Also provided is a process to obtain a plant with altered growth characteristics or altered architecture comprising the step of altering the level or functional level of the cell-division controlling protein by integrating a chimeric gene into the genome of the cells of the plant, comprising the following operably linked DNA fragments:

a) a plant expressible promoter region, particularly a CaMV35S promoter region,

b) a transcribed DNA region encoding an RNA or a protein, which when expressed, either increases or decreases the level or the functional level of the cell-division controlling protein; and optionally

c) a 3' end formation and polyadenylation signal functional in plant cells.

In accordance with the invention, the transcribed DNA region encodes an antisense RNA, a ribozyme, or a sense RNA strand which when expressed reduces, inhibits or prevents the expression of a cell-division controlling protein, particularly an endogenous D-type cyclin.

Further in accordance with the invention the transcribed DNA encodes a cell-division controlling protein capable of binding the pocket domain of an Rb-like protein, preferably a cell-division controlling protein comprising an LxCxE binding motif, more preferably a D-type cyclin, particularly a D-type cyclin from plants, more particularly a D-type cyclin is selected from group of *Arabidopsis thaliana* CYCD1, *Arabidopsis thaliana* CYCD2, *Arabidopsis thaliana* CYCD3, *Nicotiana tabacum* CYCD2;1, *Nicotiana tabacum* CYCD3;1, *Nicotiana tabacum* CYCD3;2, *Helianthus tuberosus* CYCD1;1, *Zea mays* CYCD2 and *Helianthus tuberosus* CYCD3;1.

Also in accordance with the invention the transcribed RNA encodes a protein or peptide which, when expressed, increases said functional level of said cell division controlling protein, particularly a protein or peptide selected from: a mutant D-type cyclin, a part of a D-type cyclin, a D-type cyclin which has a mutation in the cyclin box, a D2-type cyclin which has a substitution of amino acid 185 or amino acid 155, a D2-type cyclin which has mutation E185A or K155A, a D-type cyclin wherein the PEST sequences are removed, a D-type cyclin wherein the LxCxE binding motif has been changed or deleted, or a D-type cyclin wherein the C-residue from the LxCxE binding motif has been deleted.

It is also an object of the invention to provide such chimeric genes.

Further provided are plant cells, plants and seed thereof, comprising the chimeric genes of the invention and having altered growth characteristics and/or altered architecture.

Another object of the invention is to provide the use of a cell-division controlling protein, capable of binding the pocket domain of an Rb-like protein and/or capable of phosphorylating an Rb-like protein, particularly a cell-division controlling protein comprising an LxCxE binding motif in the N-terminal part of the protein, more particularly a D-type cyclin and genes encoding same, to alter the growth characteristics or architecture of a plant. The cell-division controlling protein is preferably encoded by a chimeric gene, integrated in the genome of the cells of a plant.

DETAILED DESCRIPTION OF THE INVENTION

As used herein "architecture" of a plant refers to the general morphology as defined by the relative sizes, positions and number of the several parts of a plant (i.e., organs such as but not limited to leaves, inflorescences, storage organs such as tubers, roots, stems, flowers, or parts of organs such as petals, sepals, anthers, stigma, style, petiole and the like). "Altering the architecture of a plant" thus refers to changes in the general

morphology as the result of changing e.g., the number, size and position of organs or parts of organs. It is clear that altering either one organ or part of an organ or several organs or parts of organs, as described, will result in an altered plant architecture. This can be achieved by altering (i.e., enhancing or reducing) cell division activity in existing meristems and/or organ primordia or by creating *de novo* meristems.

As used herein, "co-suppression" refers to the process of transcriptional and/or post-transcriptional suppression of RNA accumulation in a sequence specific manner, resulting in the suppresion of expression of homologous endogenous genes or transgenes. Suppressing the expression of an endogenous gene can be achieved by introduction of a transgene comprising a strong promoter operably linked to a DNA region whereby the resulting transcribed RNA is a sense RNA comprising a nucleotide sequence which is has at least 75%, preferably at least 80%, particularly at least 85%, more particularly at least 90%, especially at least 95% to the coding or transcribed DNA sequence (sense) of the gene whose expression is to be suppressed. Preferably, the transcribed DNA region does not code for a functional protein. Particularly, the transcribed region does not code for a protein.

As used herein, the term "plant-expressible promoter" means a promoter which is capable of driving transcription in a plant cell. This includes any promoter of plant origin, but also any promoter of non-plant origin which is capable of directing transcription in a plant cell, e.g., certain promoters of viral or bacterial origin such as the CaMV35S or the T-DNA gene promoters.

The term "expression of a gene" refers to the process wherein a DNA region under control of regulatory regions, particularly the promoter, is transcribed into an RNA which is biologically active i.e., which is either capable of interaction with another nucleic acid or protein or which is capable of being translated into a biologically active polypeptide or protein. A gene is said to encode an RNA when the end product of the expression of the gene is biologically active RNA, such as e.g., an antisense RNA or a ribozyme. A gene is said to encode a protein when the end product of the expression of the gene is a biologically active protein or polypeptide.

The term "gene" means any DNA fragment comprising a DNA region (the "transcribed DNA region") that is transcribed into a RNA molecule (e.g., an mRNA) in a cell under control of suitable regulatory regions, e.g., a plant-expressible promoter. A gene may thus comprise several operably linked DNA fragments such as a promoter, a 5' leader sequence, a coding region, and a 3' region comprising a polyadenylation site. An endogenous plant gene is a gene which is naturally found in a plant species. A chimeric gene is any gene which is not normally found in a plant species or, alternatively, any

gene in which the promoter is not associated in nature with part or all of the transcribed DNA region or with at least one other regulatory region of the gene.

This invention is based on the unexpected finding that chimeric genes comprising DNA encoding a cell-division controlling protein capable of binding an Rb-like protein, particularly a plant cyclin of the D-type, under control of a plant-expressible promoter could be stably integrated in the genome of plant cells, without deleterious effects, and furthermore that the increased expression of such a cell-division controlling protein, particularly a cyclin of the D-type, in the plant cells led to specific alterations in the growth rate and architecture of the resulting transformed plants.

Thus, the invention relates to modulating the level of expression or activity of functional cell-division controlling proteins, preferably in a stable mannner, within plant cells of a plant to alter the architecture or the growth rate or both of the transformed plant and its progeny. Conveniently, the level or functional level of cell-division controlling proteins is controlled genetically by altering the expression of genes encoding these cell-division controlling proteins. Increasing the level or functional level of a cell-division controlling protein genetically can be achieved e.g., by manipulating the copy number of the encoding gene(s), by altering the promoter region of the encoding genes or by manipulation of the genes regulating directly or indirectly the level of the expression of a cell-division controlling protein. Alternatively, the level of a cell-division controlling protein can be increased by stabilizing the mRNA encoding the cell-division controlling protein, or by stabilizing the cell-division controlling protein e.g., by removal of destruction motifs or so-called PEST sequences.

The functional level or activity of cell-division controlling protein can be increased by the decreasing the level of an antagonist or an inhibitor of the cell-divsion promoting protein, through techniques such as, but not limited to, providing the cell with a protein, such as an inactive cell-division controlling protein similar to the one whose functional level is to be increased, or part of a such a cell-division controlling protein, which is still capable of binding an inhibitor or other regulatory protein, or is still capable of binding to cyclin-dependent kinases.The functional level or activity of cell-division controlling protein can also be increased by alteration or mutation of the cell-division controlling protein to reduce or elimate binding of an antagonist or inhibitor of the activity of the cell division related protein.

Reducing the functional level of a cell-division controlling protein can be achieved e.g., by decreasing the mRNA pool encoding the cell-division controlling protein that is available for translation, through techniques such as, but not limited to, antisense RNA, ribozyme action or co-suppresion. Alternatively, the functional level of of cell-division

controlling protein can be decreased by the increasing the level of an antagonist or an inhibitor of the cell-divsion promoting protein.

For the purpose of this invention, a "cell-division controlling protein" is a polypeptide or protein which is required for the regulation of the progression through the cell cycle of a eukaryotic cell, preferably a plant cell, or a protein which can effect the entry of cells into the cell cycle or affect progression of cells through the cell cycle by direct interaction with a protein required for the regulation of progression through the cell cycle, or a polypeptide or protein which can assume an equivalent function but is not required for the regulation of the cell cycle.

Suitable cell-division controlling proteins are proteins capable of phosphorylating either alone or in combination with other proteins an Rb-like protein, preferably capable of phosphorylating an Rb-like protein in a plant cell in the G1-S transition phase, or are capable of binding the pocket domain of retinoblastoma-like (Rb-like) proteins, preferably proteins having an LxCxE binding motif comprised within the amino-acid sequence or a related motif such as LxSxE or FxCxE (binding motifs are represented in the one-letter amino acid code wherein x represents any amino-acid). Particularly preferred are cyclins which comprise the LxCxE binding motif (and/or related motif) in the N-terminal half of the protein, preferably within the first 50 amino acid residues, particularly within the first 30 amino acid residues, such as the cyclins of the D-type, particularly plant cyclins of the D-type, especially a cyclin from the group of *Arabidopsis thaliana* CYCD1, *Arabidopsis thaliana* CYCD2, *Arabidopsis thaliana* CYCD3, *Nicotiana tabacum* CYCD3;1, *Nicotiana tabacum* CYCD2;1, *Nicotiana tabacum* CYCD3;2, *Helianthus tuberosus* CYCD1;1, *Zea mays* CYCD2 and *Helianthus tuberosus* CYCD3;1 or a cyclin with essentially similar protein sequences.

The mentioned plant cyclins of the D-type are fully characterized by the amino acid sequence encoded by the DNA sequence of EMBL Accession N° X83369 from the nucleotide position 104 to the nucleotide position 1108 *for Arabidopsis thaliana* CYCD1, EMBL Accession N° X83370 from the nucleotide position 195 to the nucleotide position 1346 *for Arabidopsis thalina* CYCD2, EMBL Accession N° X83371 from the nucleotide position 266 to the nucleotide position 1396 *for Arabidopsis thaliana* CYCD3, the nucleotide sequence of SEQ ID N° 1 from nucleotide position 182 to nucleotide position 1243 for *Nicotiana tabacum* CYCD2;1, the nucleotide sequence of SEQ ID N° 2 from nucleotide position 181 to nucleotide position 1299 for *Nicotiana tabacum* CYCD3;1, the nucleotide sequence of SEQ ID N° 3 from nucleotide position 198 to nucleotide position 1298 for *Nicotiana tabacum* CYCD3;2, the nucleotide sequence of SEQ ID N° 4 from nucleotide position 165 to nucleotide position 1109 for *Helianthus tuberosus* CYCD1;1, the nucleotide sequence of SEQ ID N° 5 from

nucleotide position 48 to nucleotide position 1118 for *Helianthus tuberosus* CYCD3;1 and the nucleotide sequence of SEQ ID N° 21 from nucleotide position 316 to nucleotide position 1389 for *Zea mays* CYCD2.

It is thought that increasing, respectively decreasing, the level or the functional level or the activity of these cell-division controlling proteins accelerates, respectively delays, the transition of G1 to the S-phase in plant cells, or increases, respectively decreases, the proportion of actively dividing cells, by their interaction with Rb-like proteins affecting the ability of the Rb-like protein to inactivate certain transcription factors. It is further thought that expression of these cell-division controlling proteins interacting with Rb-like proteins effectively allows the cells to initiate division processes, whereas (over)expression of G2/mitotic cyclins (such as cyclins of the B-type or the *cdc*25 gene product) is in contrast expected to lead to faster progression through the G2/mitotic phases of cell cycles already started.

For the purpose of this invention "Rb-like proteins" are defined as proteins from the group of human Rb-1 protein (Lee *et al.* 1987 ; Accession n° P06400), human p107 (Ewen *et al.*, 1991; Accession n° L14812) and human p130 (Hannon *et al.*1993; Accession A49370), Drosophila RBF (Du *et al.*, 1996; Accession n° for DNA entry of the encoding gene X96975), mouse RB (Bernards *et al.* 1989; Accession n° P13405) chicken RB (Boehmelt *et al.* , 1994; Accession n° X72218), *Xenopus* Rb (Destree *et al.* 1992; Accession A44879), ZmRb and Rb1 from *Zea mays* (Xie *et al.*, 1996; Grafi *et al.* 1996; Accession numbers for DNA entry of the encoding genes: X98923; GenBank U52099) as well as any protein that has simultaneously at least 25-30% amino acid sequence similarity (identity) to at least three members of the above-mentioned group, and comprises the conserved cysteine residue located at position 706 of human Rb-1 or at equivalent positions in the other Rb-like proteins (see e.g., Xie *et al.* 1996).

Rb-like proteins are members of a small family known as "pocket proteins". This term is derived from a conserved bipartite domain, the so-called "pocket domain", which is the binding site for several growth control proteins such as E2F family of transcription factors, D-type cyclins and viral oncoproteins. The A and B subdomains of the pocket domain are more conserved than the rest of the protein (~50-64% for the A and B subdomains) and are separated by a non-conserved spacer. Pocket domains are located between amino acids at positions 451 and 766 for human Rb, 321 to 811 for human p107, 438 to 962 for human p130, 445 to 758 for mouse RB, 441 to 758 for chicken RB, 440 to 767 for Xenopus Rb, 11 to 382 for corn ZmRb, 89 to 540 for corn Rb1.

For the purpose of the invention "binding to an Rb-like protein" or "binding to the pocket domain of an Rb-like protein" can be analyzed by either an *in vitro* assay or one of the *in vivo* assays, or a combination thereof. In the *in vitro* assay, the binding is analyzed between the protein in question which has been labelled by ^{35}S-methionine, and a fusion protein of glutathione-S-transferase (GST) and the pocket domain of an Rb-like protein, such as the human Rb. The fusion to GST allows easy purification and fixation of the fusion protein on glutathione sepharose beads. The interaction between the assayed protein and the Rb-like protein is compared to the binding between the same protein and a fusion protein of GST and an Rb-like protein with a mutation in the conserved cysteine at a position equivalent to cysteine 706 in human Rb, such as human Rb C706F. Such an assay has been described e.g., by Dowdy *et al.* (1993) and Ewen *et al.* (1993). In a variant of this assay, the Rb-like protein can be expressed in baculovirus-infected insect cells (Dowdy *et al.* ,1993). In a further variant, both the Rb-like protein and Rb-binding protein can be co-expressed in insect cells, and association detected by gel-filtration or co-immunoprecipitation (O'Reilly et al., 1992).

An *in vivo* assay which can be used to determine the binding of a protein to the pocket domain of Rb-like proteins, is the yeast two-hybrid system (Fields and Song, 1989). This analysis relies on the ability to reconstitute a functional GAL4 activity from two separated GAL4 fusion proteins containing the DNA binding domain ($GAL4^{BD}$) and the activation domain ($GAL4^{AD}$) fused to a pocket domain of an Rb-like protein and the protein to be assayed respectively. Expression plasmids comprising chimeric genes encoding these fusion proteins are introduced into a yeast strain encoding appropriate GAL4 inducible markers, such as strain HF7c (Feilloter *et al.*, 1994) containing GAL4-inducible *HIS*3 and *Lac*Z markers, or strain Y190 (Harper *et al.*, 1993). Proteins binding to the pocket domain of the Rb-like protein will allow growth in the absence of histidine. An example of a two-hybrid assay to demonstrate interaction of a protein with an Rb-like protein has been described by Durfee *et al.* (1993).

Preferably, suitable control experiments should be included, such as separate introduction into the same yeast strain of the expression plasmids, or introduction of expression plasmids encoding fusion proteins containing the DNA binding domain ($GAL4^{BD}$) and the activation domain ($GAL4^{AD}$) fused to a mutated pocket domain of an Rb-like protein, preferably mutated at the C706 or equivalent positions and the protein to be assayed respectively.

An alternative *in vitro* assay to determine the binding of a protein to the pocket domain of Rb-like proteins comprises transient expression of both proteins in plant cells, preferably tobacco protoplasts, and immunoprecipitation using an antibody directed against one of the two proteins to measure co-precipitation of the other protein.

For the purpose of the invention "phosphorylating an Rb-like protein" can be analyzed by an *in vitro* assay relying on the use of gamma ^{32}P-labeled adenosine-triphosphate to monitor the capacity of a protein (or a combination of proteins such as cyclins and cyclin dependent kinases) to transfer the labeled phosphate group to a target protein, as known in the art.

For the purpose of the invention "cyclin" can be defined as a regulatory protein, comprising a protein domain of about 100 amino acids known as the "cyclin box". The cyclin box is the binding site for cyclin-dependent kinases, allowing the cyclin to exert its regulatory effect on the kinase activity of the CDKs.

A cyclin box can be identified by comparing the amino acid sequence of the protein with known cyclin boxes, such as the amino acid sequence between positions 81-186 of CYCD1 from *Arabidopsis thaliana*, between positions 96-201 of CYCD2 from *Arabidopsis thaliana*, between positions 86-191 of CYCD3 from *Arabidopsis thaliana*, the cyclin boxes described by Renaudin *et al.* (1994; 1996), by Soni *et al.* (1995), and by Hemerly *et al.* (1992). An amino acid sequence identified as a cyclin box on the basis of sequence comparison should posses at least the five conserved residues required for cyclin activity R(97), D(126), L (144), K(155), E(185) (indicated positions are from the sequence of CYCD2 from *Arabidopsis thaliana*) at equivalent positions. (see *e.g.*, Soni *et al.* (1995) and Renaudin *et al.* (1996).

D-type cyclins (cyclin D or CycD) are cyclins that are characterized by the presence of additional characteristic sequences, such as the LxCxE motif or related motifs for binding Rb-like proteins, which is found within the N-terminal part of the protein, preferably located between the N-terminus and the cyclin box, particularly within the first 50 amino acids, more particularly within the first 30 amino acids of the initiating methionine-residue. Preferably, the leucine of the binding motif is preceded at position -1 or -2 by an amino acid with an acidic side chain (D, E). Alternative binding motifs such as LxSxE or FxCxE can be found. Indeed, Phelps *et al.* (1992) have identified that mutating the binding motif LxCxE in human papillomavirus E7 to LxSxE does not affect the ability of the protein to bind Rb-like proteins. Three groups of D-type cyclins have been identified on the basis of sequence homology: CycD1 (comprising *Arabidopsis thaliana* CycD1 and *Helianthus tuberosus* CYCD1;1) CycD2 (comprising *Arabidopsis thaliana* CYCD2, *Nicotiana tabacum* CYCD2;1, *Zea mays* CYCD2), CycD3 (comprising *Arabidopsis thaliana* CYCD3, *Nicotiana tabacum* CYCD3;1, *Nicotiana tabacum* CYCD3;2, and *Helianthus tuberosus* CYCD3;1).

Nomenclature and consensus sequences for the different types and groups of plant cyclins, including cyclins of the D-type, have been described by Renaudin *et al.* (1996) and can be used to classify new cyclins based on their amino acid sequence.

For the purpose of the invention, the cell-division controlling proteins can be provided to the cells either directly, e.g., by electroporation of the protoplasts in the presence of the cell-division controlling proteins, or indirectly, by transforming the plant cells with a plant-expressible chimeric gene encoding the protein to be tested either transiently, or stably integrated in the genome of the protoplasts.

In one aspect of the invention the level or the functional level of the cell-division controlling protein, capable of phosphorylating an RB-like protein or binding the pocket domain of an Rb-like proteins, is increased, to obtain a plant with altered growth rate or architecture, by integrating a chimeric gene into the genome of the cells of the plant, comprising the following operably linked DNA fragments:

a) a plant-expressible promoter region, particularly a CaMV35S promoter region,

b) a transcribed DNA region encoding a protein, which when expressed, increases the level or the functional level of the cell-division controlling protein; and optionally

c) a 3' end formation and polyadenylation signal functional in plant cells.

In a preferred embodiment of the invention, the expression level of cyclin D is increased by introduction into the genome of a plant cell, a chimeric gene comprising a transcribed DNA region encoding a cyclin D, under control of a plant-expressible promoter. The transcribed DNA region preferably comprises a nucleotide sequence selected from the nucleotide sequence of EMBL Accession N° X83369 from the nucleotide position 104 to the nucleotide position 1108, the nucleotide sequence of EMBL Accession N° X83370 from the nucleotide position 195 to the nucleotide position 1346, the nucleotide sequence of EMBL Accession N° X83371 from the nucleotide position 266 to the nucleotide position 1396, the nucleotide sequence of SEQ ID N° 1 from nucleotide position 182 to nucleotide position 1243, the nucleotide sequence of SEQ ID N° 2 from nucleotide position 181 to nucleotide position 1299, the nucleotide sequence of SEQ ID N° 3 from nucleotide position 198 to nucleotide position 1298, the nucleotide sequence of SEQ ID N° 4 from nucleotide position 165 to nucleotide position 1109, the nucleotide sequence of SEQ ID N° 5 from nucleotide position 48 to nucleotide position 1118 or the nucleotide sequence of SEQ ID N° 21 from nucleotide position 316 to nucleotide position 1389 for *Zea mays* CYCD2.

In a particularly preferred embodiment the expression level of a cyclin of the CycD2 type is altered (i.e., increased) by introduction into the genome of a plant cell, of a "chimeric *cyc*D2 gene" comprising a transcribed DNA region encoding a cyclin of the

CycD2 type, under control of a plant-expressible promoter, preferably a constitutive promoter, particularly a CaMV35S promoter, such as the chimeric *cyc*D2 gene of plasmid pCEC1, in order to alter the morphology, architecture and growth characteristics of the transgenic plant, particularly to increase the vegetative growth of the transgenic plant, more particularly to alter the growth rate of the transgenic plant.

For the purpose of the invention, "increase" or "decrease" of a measurable phenotypic trait is quantified as the difference between the mean of the measurements pertinent to the description of that trait in different plants of one transgenic plant line, and the mean of the measurements of that trait in wild type plants, divided by the mean of the measurements of that trait in wild type plants, expressed in percentage, whereby transgenic and control (wild type) plants are grown under the same conditions of nutrient supply, light, moisture, temperature and the like, preferably under standardized conditions. Prefered levels of increase or decrease are statistically significant, preferably at the 0.05 confidence level, particularly at the 0.01 confidence level, e.g., by one way variance analysis (e.g., as described in *Statistical Methods* by Snedecor and Cochran).

Increase of the vegetative growth of a transgenic plant is preferably monitored by measuring the increase in dry weight during the growth period. The mean increase of dry weight is defined as the difference in mean dry weight of transgenic plants and wild type plants multiplied by 100 and divided by the mean dry weight of wild type plants. Typical increases in dry weight, particularly early in growth period, by introduction of the chimeric *cyc*D2 genes of the invention range from at least about 39% to about 350%, particularly from about 68% to about 150%.

It is clear that increases in dry weight resulting from introduction of the chimeric genes of the invention may vary, depending on the plant species or chimeric genes used, and any significant increase in dry weight in transgenic plants is encompassed by the invention, particularly a dry weight of at least about 1.4 times to at least about 4.5 times the dry weight in untransformed control plants, particularly of at least about 1.8 times to at least about 2.7 times the dry weight in untransformed control plants. In any case, the mean dry weight of the transgenic plants is statistically significantly different from the mean dry weight of the untransformed plants.

Increase in the vegetative growth of a transgenic plant can also be determined by comparing the number of leaves visible on the transgenic plants and the control wild-type plants at any given point in time. The difference in number of leaves of transgenic plants in the middle of the growth period is expected to be at least about 1.1 to at least

about 3 times, particularly at least about 1.5 to at least about 2 times the leaf number in untransformed plants.

Increase of the vegetative growth of a transgenic plant can also be monitored by measuring the height of the stem (measured from soil level to the top of growing point) during the growth period. The mean increase of the stem height is defined as the difference in mean stem height of transgenic plants and wild type plants multiplied by 100 and divided by the mean height of wild type plants. Typical increases in stem height by introduction of the chimeric *cyc*D2 genes of the invention range from at least about 65% early during growth, over at least about 20-30% in the middle of the growing period, to at least about 10% by the time of flowering, but may be as high as about 120% to about 190% early during growth, as high as about 40-50% to about 75% in the middle of the growing period, and as high as about 15-20% at the end of the flowering stage.

It is clear that increases in stem height resulting from introduction of the chimeric genes of the invention may vary, depending on the plant species or chimeric genes used, and any significant increase in stem height in transgenic plants is encompassed by the invention, particularly stem height of at least about 1.1 times to at least about 3 times the stem height in untransformed control plants, particularly of at least about 1.5 times to at least about 2 times the stem height in untransformed control plants.

The difference in stem height between transgenic and control plants diminishes as growth progresses, because the growth rate slows down in plants that are flowering. The terminal height of a transgenic plant, may thus be similar to the terminal height of a non-transgenic plant.

The transgenic plants comprising the chimeric *cyc*D2 genes of the invention have an increased growth rate, when compared with untransformed plants, resulting in a reduced time required to reach a given dry weight or stem height. "Growth rate" as used herein, refers to the increase in size of a plant or part of plant per day, particularly to increase in stem height per day, and can be calculated as the difference between the size of a plant or part of a plant at the start and end of a period comprising a number of days, particularly 6 to 8 days, divided by the number of days. Increase in growth rate is preferably expressed according to the general definition of increase of a measurable phenotype, but can also be expressed as the ratio between the growth rate of the transgenic plants, versus the growth rate of the untransformed control plants, during the same period, under the same conditions.

As mentioned before the increase in growth rate resulting from introduction of the chimeric genes, particularly the chimeric *cyc*D2 genes of the invention may vary, depending on the plant species or the chimeric genes used, and any significant increase in growth rate in transgenic plants is encompassed by the invention, particularly increase in growth rate ranging from about 4% to about 85%, more particularly from about 20% to about 60%, especially from about 30% to about 50%.

Increase of the vegetative growth of a transgenic plant can also be monitored by measuring the length or the size of the largest leaf at different time points during the growth period whilst the leaves are still expanding. This measurable phenotype is a measure of the increased maturity of the transgenic plants. The mean increase of the length of the largest leaf (defined as the difference between mean length of the largest leaf of transgenic plants and wild type plants multiplied by 100 and divided by the mean length of the largest leaf of wild type plants) obtained by introduction of the chimeric genes of the invention ranges from about 7 to 31% (mean about 17%) early during growth, to about 3-14% (mean about 7%) in the middle of the growing period.

Again, these increases in the size of the largest leaf, resulting from introduction of the chimeric genes of the invention, may vary, depending on the plant species or chimeric gene used, and any significant increase in leaf growth or size in transgenic plants is encompassed by the invention.

As another object of the invention, the chimeric cell-division controlling gene, particularly the chimeric *cyc*D2 genes, can also be introduced in plants to increase the root development, particularly to increase the mean root length. In general, the increase in root development, is parallel to the increase in the vegetative part above the ground (stem, leaves, flowers) and may range from about 40% to about 70%, but again these increases may vary depending on the plant species or chimeric gene used, and any significant increase, particularly statistically significant increase in root development is encompassed by the invention.

As yet another object of the invention, the chimeric cell-division controlling gene, particularly the chimeric *cyc*D2 genes, can also be introduced in plants to increase the size as well as the number of flowers, particularly the number of fertilized flowers, and the number of fertilized ovules in each flower . As a result of the increase in the number of fertilized flowers, and the number of fertilized ovules in each flower (generally leading to a greater number of seeds per plant), it is clear that also an increase in seed yield per plant can be obtained. It is clear that the increase in the number of flowers and ovules per flower, as well as the increase in seed yield can vary, depending on the plant species transformed with the chimeric cell-division controlling genes of the

invention or the chimeric genes used. Typical increases in flower size resulting from the introduction of a chimeric gene comprising a CycD2 encoding DNA region under control of a CaMV35S promoter range from at least about 4% to at least about 30%, particularly at least about 10% to at least about 20%. Typical increases in the number of flowers range from about at least 20% to at least about 50%, particularly from about 24% to about 45% while increases in the number of seeds/ plants (expressed on a weight basis) are in a range from at least about 5% to at least about 55%, particularly from at least about 10% to at least about 30%, more particularly about 25%.

In still another embodiment of the invention, the chimeric cell-division controlling gene, particularly the chimeric *cyc*D2 genes, can also be introduced in plants or their seeds to accelerate germination. It has been found that transgenic seeds comprising the chimeric *cyc*D2 genes of the invention can germinate at least between about 8 to about 16 hrs faster than wild type controls.

Moreover, the mentioned chimeric genes can also be introduced in plants to decrease the mean number of days required to reach the development of an inflorescence, thus effectively reducing the time required to start flowering. Transgenic plants comprising the chimeric *cyc*D2 genes of the invention thus reach maturity, particularly the flowering stage, earlier, but have the normal size of a flowering plant. The actual reduction in time required to reach the flowering stage may depend on the plant species or chimeric genes used. Typically, transgenic plants harboring the chimeric gene comprising a CycD2 encoding DNA region under control of a CaMV35S promoter exhibit a reduction in the time required to flower of at least about 3% to 11 -12%, particularly at least about 4% to 7%.

In another particularly preferred embodiment, a chimeric gene comprising a CycD3 encoding transcribed DNA region under control of a plant-expressible promoter, preferably a constitutive promoter, particularly a CaMV35S promoter, such as a chimeric gene comprising the nucleotide sequence of the chimeric *cyc*D3 gene of pCRK9, is introduced into a plant cell to obtain transgenic plants with altered morphological traits or architecture, particularly with altered size of specific plant parts or organs, more particularly with altered flower size and morphology such as flowers with elongated and/or enlarged petals. Transgenic plants transformed with a chimeric gene comprising a CycD3 encoding DNA region under control of a plant-expressible promoter (and the progeny thereof) exhibit an increase in the flower size of about 31% to about 44%. Moreover these transgenic plants also flower later than wild type plants, corresponding to an increase in flowering time of about 5 % to about 20%, particularly about 8% to about 16%.

In another embodiment of the invention the functional level of the cell-division controlling protein, capable of phosphorylating an RB-like protein or binding the pocket domain of an Rb-like proteins, particularly of the D-type cyclin is increased, to obtain a plant with altered growth rate or architecture, by integrating a chimeric gene into the genome of the cells of the plant, comprising the following operably linked DNA fragments:

a) a plant-expressible promoter region, particularly a CaMV35S promoter region,

b) a transcribed DNA region encoding a protein, which when expressed, increases the functional level of a cell-division controlling protein, preferably encoding a mutant cell-division controlling protein or part of a mutant cell-division controlling protein, more preferably encoding a mutant D-type cyclin or part of a D-type cyclin, particularly encoding a D-type cyclin which has a mutation in cyclin box (quite particularly a substitution of amino acid 185 or amino acid 155 of a D2-type cyclin, especially E185A or K155A), or a D-type cyclin wherein the PEST sequences are removed, particularly which has been C-terminally deleted to remove the PEST sequences, or a D-type cyclin wherein the LxCxE binding motif has been changed or deleted, particularly wherein the C-residue from the LxCxE binding motif has been deleted; and optionally

c) a 3'end formation and polyadenylation signal functional in plant cells.

Although not intending to limit the invention to a mode of action, it is believed that the mutant cell-division controlling proteins exert their effects by sequestering inhibitors or antagonists of the normal functional cell-division controlling proteins.

It is clear from this description that chimeric genes comprising a transcribed DNA region encoding other cyclins of the D-type, particularly plant-derived cyclins of the CycD group, may be used to obtain similar effects. These genes can be obtained from other plant species or varieties, by different methods including hybridization using the available CycD1, CycD2 or CycD3 encoding DNAs as probes and hybridization conditions with reduced stringency, or polymerase chain reaction based methods using oligonucleotides based on the available nucleotide sequences of D-type cyclins, preferably oligonucleotides having a nucleotide sequence corresponding to the sequences encoding the consensus amino acid sequences, particularly oligonucleotides having a nucleotide sequence corresponding to the sequences encoding conserved amino acid sequences within the cyclin box for each group of cyclins. These conserved amino acid sequences can be deduced from available aligned DNA encoding such amino acid sequences. A particularly preferred combination of oligonucleotides for PCR amplification of plant cyclins of the D1 type is an oligonucleotide selected from the group of oligonucleotides having the DNA sequence of SEQ ID N° 7, SEQ ID N° 8 or SEQ ID N° 9 and an oligonucleotide

selected from the group of oligonucleotides having the DNA sequence of SEQ ID N° 10 or SEQ ID N° 11.

A particularly preferred combination of oligonucleotides for PCR amplification of plant cyclins of the D2 type is an oligonucleotide selected from the group of oligonucleotides having the DNA sequence of SEQ ID N° 12 or SEQ ID N° 13 and an oligonucleotide selected from the group of oligonucleotides having the DNA sequence of SEQ ID N° 14 or SEQ ID N° 15. A particularly preferred combination of oligonucleotides for PCR amplification of plant cyclins of the D3 type is an oligonucleotide selected from the group of oligonucleotides having the DNA sequence of SEQ ID N° 16, SEQ ID N° 17 or SEQ ID N° 18 and an oligonucleotide selected from the group of oligonucleotides having the DNA sequence of SEQ ID N° 19 or SEQ ID N° 20. The amplified DNA fragment is then used to screen a cDNA or genomic library (under stringent conditions) to isolate full length clones.

Alternatively, additional genes encoding plant-derived cyclins can be obtained by techniques such as, but not limited to, functional complementation of conditional G1-S cyclin deficient yeast strains, as described by Soni *et al.* (1995) and Dahl *et al.* (1995) or by using the yeast two-hybrid system (Fields and Song, 1989) to isolate DNA sequences encoding cyclins binding to the pocket domain of Rb-like proteins as described *supra*.

It is further known that some plants contain more than one gene encoding a D-type cyclin of the same subgroup (e.g., tobacco contains at least two genes of the CycD3 subgroup) and it is clear that these variants can be used within the scope of the invention.

Moreover D-type cyclins which have an amino acid sequence which is essentially similar to the ones disclosed in this invention, such as mutant D-type cyclins, can be used to the same effect. With regard to "amino acid sequences", essentially similar means that when the two relevant sequences are aligned, the percent sequence identity -i.e., the number of positions with identical amino acid residues divided by the number of residues in the shorter of the two sequences- is higher than 80%, preferably higher than 90%. The alignment of the two amino acid sequences is performed by the Wilbur and Lipmann algorithm (Wilbur and Lipmann ,1983) using a window-size of 20 amino acids, a word length of 2 amino acids, and a gap penalty of 4. Computer-assisted analysis and interpretation of sequence data, including sequence alignment as described above, can be conveniently performed using the programs of the Intelligenetics™ Suite (Intelligenetics Inc., CA).

It is clear that any DNA sequence encoding a cell-division controlling protein, particularly a D-type cyclin, can be used to construct the chimeric cell-division controlling genes of the invention, especially DNA sequences which are partly or completely synthesized by man.

It is also clear that other plant-expressible promoters, particularly constitutive promoters, such as the the opine synthase promoters of the Agrobacterium Ti- or Ri-plasmids, particularly a nopaline synthase promoter can be used to obtain similar effects. Moreover, in the light of the existence of variant forms of the CaMV35S promoter, as known by the skilled artisan, the object of the invention can be equally be achieved by employing these alternative CaMV35S promoters, such as those described by Hull and Howell, *Virology*, **86**, pg. 482 (1978).

It is a further object of the invention to provide plants with altered morphology or architecture, restricted to specific organs or tissues by using tissue-specific or organ-specific promoters to control the expression of the DNA encoding a cell-division controlling protein, particularly a cyclin of the D-type. Such tissue-specific or organ-specific promoters are well known in the art and include but are not limited to seed-specific promoters (e.g., WO89/03887), organ-primordia specific promoters (An *et al.*, 1996), stem-specific promoters (Keller *et al.*, 1988), leaf specific promoters (Hudspeth *et al.* ,1989), mesophyl-specific promoters (such as the light-inducible Rubisco promoters), root-specific promoters (Keller *et al.*,1989), tuber-specific promoters (Keil *et al.*, 1989), vascular tissue specific promoters (Peleman *et al,*. 1989), meristem specific promoters (such as the promoter of the *SHOOTMERISTEMLESS* (*STM*) gene, Long *et al.*, 1996), primordia specific promoter (such as the promoter of the *Antirrhinum* CycD3a gene, Doonan *et al.* , 1998) and the like.

In another embodiment of the invention, the expression of a chimeric gene encoding a cell-division controlling protein can be controlled at will by the application of an appropriate chemical inducer, by operably linking the DNA region coding for the cell-division controlling protein to a promoter whose expression is induced by a chemical compound, such as the promoter of the gene disclosed in European Patent publication "EP" 0332104, or the promoter of the gene disclosed in W0 90/08826.

In yet another embodiment of the invention, the expression of a chimeric gene encoding a cell-division controlling protein can be controlled by use of site-specific recombinases and their corresponding *cis*-acting sequences, e.g., by inserting between the plant-expressible promoter and the transcribed region encoding the cell-division controlling protein, an unrelated nucleotide sequence (preferably with transcriptional and/or translational termination signals) flanked by the cis-acting sequences recognized by a

site-specific recombinase (e.g., *lox* or FRT sites); providing the plant cells comprising this chimeric gene with the site-specific recombinase (e.g., Cre or FLP) so that the inserted unrelated nucleotide sequence is eliminated by recombination, thus allowing the chimeric cell division controlling gene to be expressed.

It is thought that the morphological alterations obtained by increased expression of cell-division controlling proteins, particularly D-type cyclins in plants due to the introduction of a chimeric gene comprising a DNA region encoding a cell-division controlling protein, particularly a D-type cyclin under control of a plant expressible promoter, can be enhanced, by removal, adaptation or inactivation of PEST sequences. PEST sequences are amino acid sequences which are rich in proline, glutamate or aspartate and serine or threonine, located between positively charged flanking residues, which are involved in rapid turnover of the protein comprising such sequences (Tyers *et al.* , 1992; Cross, 1988; Wittenberg and Reed, 1988; Salama *et al.*, 1994). Removal of these PEST sequences in yeast cyclins stabilizes the cyclins *in vivo* (Pines, 1995). PEST regions can be identified by computer analysis, using software packages such as PESTFIND (Rogers *et al.* ,1986; Rechsteiner, 1990). Mutation of a DNA encoding cell-division controlling protein with altered PEST sequences is well within the reach of the skilled artisan using methods such as described e.g., by Sambrook *et al.* (1989)

It is further expected that the quantitative effects of phenotypic alterations can be modulated -i.e., enhanced or repressed- by expression of endogenous cell-division controlling encoding chimeric genes, particularly endogenous CycD encoding chimeric genes as an alternative to using heterologous genes encoding similar proteins from other plants. Preferably, heterologous genes are used, particularly heterologous genes encoding similar proteins with less than about 65% , preferably less than about 75%, more preferably less than about 65% amino acid sequence identity to the endogenous cell division controlling protein.

In another aspect of this invention, the morphology of plants can be altered by decreasing expression of a functional cell-division controlling protein, particularly a D-type cyclin. This can be achieved using e.g., antisense-RNA, ribozyme, or co-suppresion techniques. To this end, a chimeric gene comprising a transcribed DNA region which is transcribed into an RNA, the production of which reduces, inhibits or prevents the expression of a cell-division controlling protein, particularly a D-type cyclin within the plant cells is introduced in the plant cells, particularly stably integrated in the genome of the plant cells.

In one embodiment of this aspect, the transcribed DNA region of the chimeric gene encodes an antisense RNA which is complementary to at least part of a sense mRNA

encoding a cell-division controlling protein, particularly a D-type cyclin. The antisense RNA thus comprises a region which is complementary to a part of the sense mRNA preferably to a continuous stretch thereof of at least 50 bases in length, particularly of at least between 100 and 1000 bases in length. The antisense RNA can be complementary to any part of the mRNA sequence: it may be complementary to the sequence proximal to the 5' end or capping site, to part or all of the leader region, to an intron or exon region (or to a region bridging an exon and intron) of the sense pre-mRNA, to the region bridging the noncoding and coding region, to all or part of the coding region including the 3' end of the coding region, and/or to all or part of the 3' or trailer region. The sequence similarity between the antisense RNA and the complement of the sense RNA encoding a cell-division controlling protein, should be in the range of at least about 75% to about 100%.

In another embodiment of this aspect, the transcribed DNA region of the chimeric gene encodes a specific RNA enzyme or so-called ribozyme (see e.g., WO 89/05852) capable of highly specific cleavage of the sense mRNA encoding a cell-division controlling protein, particularly a D-type cyclin.

In yet another embodiment, the level of a functional cell-division controlling protein, particularly a D-type cyclin can be decreased by the expression of chimeric gene comprising a DNA region encoding a protein or polypeptide which when expressed reduces the level of a cell-division controlling protein, particularly a D-type cyclin, or inhibits the cell division controlling protein, particularly the D-type cyclin, to exert its function within the plant cells. Preferably, the chimeric gene encodes an antibody that binds to a cell-division controlling protein, particularly a D-type cyclin.

Decreasing the level or the functional level of a cell-division controlling protein, particularly a D-type cyclin within the cells of a transgenic plant, comprising the chimeric genes of this embodiment of the invention, results in altered architecture, particularly in a decreased stem height, a decrease of the growth rate or a delaying in the flowering of the transgenic plants when compared to untransformed plants, grown under the same conditions. The effect obtained might vary, depending on the plant species or chimeric genes used, and any effect on architecture and/or growth rate, particularly a decrease in stem height or growth rate, or an increase in the time required to develop an inflorescence, is encompassed by the invention.

The decrease in growth rate due to decreasing the level of a cell-division controlling protein, preferably a D-type cyclin, particularly a CYCD2 type cyclin, ranges from about 30% to about 60%, particularly from about 35% to about 50%.

The decrease in stem height due to decreasing the level of a cell-division controlling protein, preferably a D-type cyclin, particularly a CYCD2 type cyclin, ranges from about 10% to about 60%, particularly from about 30% to about 50%, more particularly around 40%.

The increase in flowering time due to decreasing the level of a cell-division controlling protein, preferably a D-type cyclin, particularly a D2 type cyclin, ranges from about 10% to about 40%, particularly from about 15% to about 38%.

The chimeric cell-division controlling gene may include further regulatory or other sequences, such as leader sequences [e.g., cab22L leader from Petunia or the omega leader from TMV (Gallie *et al.*, 1987)], 3' transcription termination and polyadenylation signals (e.g., of the octopine synthase gene [De Greve *et al.*, 1982)], of the nopaline synthase gene [Depicker *et al.*,1982] or of the T-DNA gene 7 [Velten and Schell, 1985] and the like [Guerineau *et al.*, 1991; Proudfoot ,1991; Safacon *et al.*, 1991; Mogen *et al.*, 1990; Munroe *et al.*, 1990; Ballas *et al.*, 1989; Joshi *et al.*,1987], plant translation initiation consensus sequences [Joshi, 1987], introns [Luehrsen and Walbot, 1991] and the like, operably linked to the nucleotide sequence of the chimeric cell-division controlling gene.

Preferably, the recombinant DNA comprising the chimeric cell-division controlling gene is accompanied by a chimeric marker gene. The chimeric marker gene can comprise a marker DNA that is operably linked at its 5' end to a plant-expressible promoter, preferably a constitutive promoter, such as the CaMV 35S promoter, or a light inducible promoter such as the promoter of the gene encoding the small subunit of Rubisco; and operably linked at its 3' end to suitable plant transcription 3' end formation and polyadenylation signals. It is expected that the choice of the marker DNA is not critical, and any suitable marker DNA can be used. For example, a marker DNA can encode a protein that provides a distinguishable color to the transformed plant cell, such as the A1 gene (Meyer *et al.*, 1987), can provide herbicide resistance to the transformed plant cell, such as the *bar* gene, encoding resistance to phosphinothricin (EP 0,242,246), or can provided antibiotic resistance to the transformed cells, such as the *aac(6')* gene, encoding resistance to gentamycin (WO94/01560).

Although it is clear that the invention can be applied essentially to all plant species and varieties, the invention will be especially suited to alter the architecture or to increase the growth rate of plants with a commercial value. It is expected that the enhancements in vegetative growth will be most pronounced in plants which have not undergone extensive breeding and selection for fast vegetative growth. The invention will be particularly relevant for plants which are grown in greenhouses, particularly to reduce

the time required for greenhouse plants to reach the desired developmental stage, such as but not limited to flowering, fruit setting or seed setting. The invention will further be relevant to enhance the growth rate of trees, particularly softwood trees such as pine, poplar, *Eucalyptus* trees and the like. Another important application of the invention encompasses the expansion of effective area wherein plants can be cultivated by reduction of the time required to reach the economically important developmental stage. Particularly preferred plants to which the invention can be applied are corn, oil seed rape, linseed, wheat, grasses, alfalfa, legumes, a brassica vegetable, tomato, lettuce, rice, barley, potato, tobacco, sugar beet, sunflower, and ornamental plants such as carnation, chrysanthemum, roses, tulips and the like.

A recombinant DNA comprising a chimeric cell-division controlling gene can be stably incorporated in the nuclear genome of a cell of a plant. Gene transfer can be carried out with a vector that is a disarmed Ti-plasmid, comprising a chimeric gene of the invention, and carried by *Agrobacterium*. This transformation can be carried out using the procedures described, for example, in EP 0,116,718. Alternatively, any type of vector can be used to transform the plant cell, applying methods such as direct gene transfer (as described, for example, in EP 0,233,247), pollen-mediated transformation (as described, for example, in EP 0,270,356, WO85/01856 and US 4,684,611), plant RNA virus-mediated transformation (as described, for example, in EP 0,067,553 and US 4,407,956), liposome-mediated transformation (as described, for example, in US 4,536,475), and the like.

Other methods, such as microprojectile bombardment as described, for corn by Fromm *et al.* (1990) and Gordon-Kamm *et al.* (1990), are suitable as well. Cells of monocotyledonous plants, such as the major cereals, can also be transformed using wounded and/or enzyme-degraded compact embryogenic tissue capable of forming compact embryogenic callus, or wounded and/or degraded immmature embryos as described in WO92/09696.The resulting transformed plant cell can then be used to regenerate a transformed plant in a conventional manner.

The obtained transformed plant can be used in a conventional breeding scheme to produce more transformed plants with the same characteristics or to introduce the chimeric cell-division controlling gene of the invention in other varieties of the same or related plant species. Seeds obtained from the transformed plants contain the chimeric cell-division controlling gene of the invention as a stable genomic insert.

The following non-limiting Examples describe the construction of chimeric cell-division controlling genes and the use of such genes for the modification of the architecture and growth rate of plants. Unless stated otherwise in the Examples, all recombinant

DNA techniques are carried out according to standard protocols as described in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, NY and in Volumes 1 and 2 of Ausubel *et al.* (1994) *Current Protocols in Molecular Biology, Current Protocols*, USA. Standard materials and methods for plant molecular work are described in *Plant Molecular Biology Labfax* (1993) by R.D.D. Croy, jointly published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications, UK.

Throughout the description and Examples, reference is made to the following sequences:

SEQ ID N° 1: cDNA encoding *Nicotiana tabacum* CYCD2;1
SEQ ID N° 2: cDNA encoding *Nicotiana tabacum* CYCD3;1
SEQ ID N° 3: cDNA encoding *Nicotiana tabacum* CYCD3;2
SEQ ID N° 4: cDNA encoding *Helianthus tuberosus* CYCD1;1
SEQ ID N° 5: cDNA encoding *Helianthus tuberosus* CYCD3;1
SEQ ID N° 6: T-DNA of pGSV5
SEQ ID N° 7: PCR primer 1
SEQ ID N° 8: PCR primer 2
SEQ ID N° 9: PCR primer 3
SEQ ID N° 10: PCR primer 4
SEQ ID N° 11: PCR primer 5
SEQ ID N° 12: PCR primer 6
SEQ ID N° 13: PCR primer 7
SEQ ID N° 14: PCR primer 8
SEQ ID N° 15: PCR primer 9
SEQ ID N° 16: PCR primer 10
SEQ ID N° 17: PCR primer 11
SEQ ID N° 18: PCR primer 12
SEQ ID N° 19: PCR primer 13
SEQ ID N° 20: PCR primer 14
SEQ ID N° 21: cDNA encoding *Zea mays* CYCD2

Plasmids pCEC1 and pCRK9 have been deposited at the
Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Moleculaire Biologie-Plasmidecollectie (LMBP)
Universiteit Gent
K. L. Ledeganckstraat 35
B-9000 Gent, Belgium
on 11 March 1997 and have been attributed the following deposition numbers:

MC1061(pCEC1): BCCM/LMBP3657
DH5α (pCRK9): BCCM/LMBP3656

Plasmids pBlueScript-ZM18 has been deposited at the
Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Moleculaire Biologie-Plasmidecollectie (LMBP)
Universiteit Gent
K. L. Ledeganckstraat 35
B-9000 Gent, Belgium
on 19 March 1998 under the deposit number BCCM/LMBP 3866.

EXAMPLES

Example 1. Construction of the chimeric genes

1.1 Construction of the CaMV35S-AthcycD2 chimeric gene and inclusion in a T-DNA vector.

A 1298 bp *Nco*I-*Sac*I fragment comprising the DNA encoding CYCD2 from *A. thaliana* (having the nucleotide sequence of EMBL Accesion N° X83370 from nucleotide position 194 to nuceotide position 1332) was treated with Klenow polymerase to render the protruding termini blunt, and ligated to *Sma*I linearized pART7 (Gleave, 1992), yielding plasmid pCEC1. In this way, a chimeric gene flanked by *Not*I sites was constructed, wherein the DNA encoding the CYCD2 was operably linked to a CaMV35S promoter of the CabbB-J1 isolate (Harpster *et al.*,1988) and a 3'*ocs* region (MacDonald *et al.*, 1991) . The chimeric gene was then inserted between the T-DNA border of a T-DNA vector, comprising also a selectable chimeric marker gene.

To this end, the chimeric *cyc*D2 gene was excised from pCEC1, using *Not*I, and ligated to *Not*I linearized pART27 (Gleave, 1992) to create pCEC5. pART27 comprises a chimeric selectable marker gene consisting of the following operably linked fragments: a nopaline synthase gene promoter, a *neo* coding region and 3' end of a nopaline synthase gene (An *et al.*, 1988).

Alternatively, the chimeric *cyc*D2 gene is excised from pCEC1 using an appropriate restriction enzyme (e.g. *Not*I) and introduced in the polylinker between the T-DNA border sequences of the T-DNA vector pGSV5, together with a selectable chimeric marker gene (pSSU-*bar*-3'ocs; De Almeida *et al.*, 1989) yielding pCEC5b.

pGSV5 was derived from plasmid pGSC1700 (Cornelissen and Vandewiele, 1989) but differs from the latter in that it does not contain a beta-lactamase gene and that its T-DNA is characterized by the sequence of SEQ ID No 6.

1.2 Construction of the CaMV35S-AthcycD3 chimeric gene and inclusion in a T-DNA vector.

The *cyc*D3 cDNA was isolated as a 1335 bp *Bsl*I-*Dra*I fragment, rendered blunt-ended by treatment with Klenow polymerase (having the nucleotide sequence of EMBL Accesion N° X83371 from nucleotide position 104 to nucleotide position 1439) and inserted into the *Sma*I site of pUC18, to create pRS14a. This clone carries the full coding sequence of *cyc*D3, with the translation initiation codon located immediately adjacent to the cleaved *Sma*I site of pUC18 in such an orientation that the *Sac*I site of pUC18 is at the 5' end of the *cyc*D3 cDNA and the *Bam*HI site is at the 3' end. The 1.35 kb *Sac*I-*Bam*HI fragment of pRS14a was isolated and ligated to the about 26.6 kb *Sac*I-*Bam*HI fragment of pSLJ94 (Jones *et al.*, 1992), generating pCRK9. In this way a chimeric gene was constructed wherein the DNA encoding the *cyc*D3 coding region from *A. thaliana* was operably linked to a CaMV35S promoter and the 3'*ocs* region. In pCRK9 the chimeric gene is located between T-DNA borders, accompanied by a chimeric selectable *neo* gene (Jones *et al.* ,1992)

Alternatively, the chimeric *cyc*D3 gene is excised from pRS14a using appropriate restriction enzymes and introduced in the polylinker between the T-DNA border sequences of the T-DNA vector pGSV5, together with a selectable chimeric marker gene (pSSU-*bar*-3'ocs; De Almeida *et al.*, 1989) yielding pCRK9b.

Example 2. *Agrobacterium*-mediated transformation of tobacco plants with the T-DNA vectors of Example 1.

T-DNA vectors pCEC5 and pCRK9 were introduced in *Agrobacterium tumefaciens* LBA4404 (Klapwijk *et al.*, 1980) by electroporation as described by Walkerpeach and Velten (1995) and transformants were selected using spectinomycin and tetracycline respectively.

T-DNA vectors pCEC5b and pCRK9b are introduced in *A. tumefaciens* C58C1Rif^R by triparental mating (Ditta *et al.*, 1980).

The resulting *Agrobacterium* strains were used to transform *Nicotiana tabacum* var Xanthi, applying the leaf disc transformation method as described in An *et al.* (1985).

Eight tobacco plants transformed with pCRK9 (designated 1K9, 2K9, 3K9, 4K9, 8K9, 10K9, 17K9, 19K9 and 28K9) were generated and eleven tobacco plants transformed with pCEC5 (designated C8 lines 1 to 3 and 5 to 12).

Plants transformed by pCRK9 T-DNA were analyzed for the copy number of the inserted transgenes by Southern hybridization using the labelled cDNA insert of pRS14a as probe. Lines 2K9, 3K9 and 4K9 each had obtained 1 copy of the transgene, while line 1K9 contained three copies of the transgene.

Plants transformed by pCEC5 T-DNA were analyzed for the copy number of the inserted transgenes by Southern hybridization using *Bam*HI digested DNA prepared from these plants and labelled 0.7 kb *Nco*I-*Eco*RI fragment from J22 cDNA (comprising part of the *cyc*D2 coding region; Soni *et al.*, 1995). Lines C8-2, C8-3, C8-5, C8-8, C8-1, C8-9, C8-10, C8-11, C8-12 all had one copy of the transgene, line C8-7 had two copies, line C8-6 had three copies and line C8-1 had four copies of the transgene.

The T0 (primary transformants) were self-fertilized and allowed to set seeds (T1 seeds). Plants grown from T1 seeds were designated C8-T1-X, where X stands for the line number of the original transformant. Seeds from T1 plants were referred to as T2 seed; plants grown from such seed were named C8-T2-X, where X is again the line number of the original transformant. Whenever the generation was not mentioned, the plants were grown from T1 seed.

Northern analysis confirmed transcription of the transgenes in at least lines C8-1, C8-3, C8-7, 3K9, 4K9 and 8K9.

Example 3. Phenotypic analysis of the transformed tobacco plants.

3.1. Tobacco plants comprising the CaMV35S-AthCycD2 chimeric gene.

Seeds from primary transformants (T0 plants) were surface sterilized in 10% bleach for 15 minutes and thoroughly washed in sterile water. The surface-sterilized seeds were germinated on GM medium containing kanamycin to a final concentration of 100 µg/ml. Seeds on plates were placed for 5 days at 4°C (vernalization) and then moved to 23°C in a growth chamber. All time points refer to the day of placing in the growth chamber. Eighteen days after moving to the growth chamber (ie after 23 days in total), the kanamycine-resistant seedlings were transplanted into seed trays containing soil, and grown under 18 hr photoperiod in a growth room. After a further 10 days these plants were transferred to 3 inch plant pots and after an additional 15 days to 8 inch plant pots

where they remained for the rest of the experiment. The 3 inch and 8 inch plant pots were incubated in a greenhouse supplemented with additional lighting to achieve an 18 hour photoperiod. Plants were placed in randomised design within the greenhouse.

Measurements were started two days later (i.e., after 45 days or after 27 days in soil; referred to as week 1), and repeated every week for seven weeks, when appropriate. The following number of plants were analyzed for each line: 22 plants for line C8-1, 7 plants for line C8-2, 22 plants for line C8-3, 8 plants for line C8-5, 6 plants for line C8-6, 22 plants for line C8-7, 5 plants for line C8-8, 6 plants for line C8-9, 4 plants for line C8-10, 6 plants for line C8-11, 5 plants for line C8-12, 34 plants for untransformed control (wild type).

The folllowing parameters were analyzed : height of the plants from the soil surface to the highest point (*i.e.,* growing tip; summarized in Table 1 as mean height ± standard deviation in cm); length of the largest leaf at defined times (summarized in Table 2 as mean length ± standard deviation in cm); time (summarized in Table 3 as mean time ± standard deviation in days) at which an infloresence meristem is visible with the naked eye (inflorescences of 0.25cm and 1 cm); height at which an infloresence meristem is visible (summarized in Table 3 as mean length ± standard deviation in cm); length of the petal tube of the flowers; width of the collar of the petal tube (summarized in Table 3 as mean length and width ± standard deviation in mm); total number of seed pods per plant; and average seed yield (on a weight basis) per plant.

The transgenic plants exhibited an increased growth rate, apparent from the seedling stage, resulting in a larger average stem height (Table 1). At time point week 3, all populations of transgenic lines are significantly larger than the untransformed controls (t-test; at confidence level 95%), while lines C8-1, C8-2, C8-3, C8-5, C8-11 are significantly larger than the untransformed controls at a confidence level of 99%. The increased growth rate also resulted on average in larger leaves at the indicated times, which correspond to a period when leaf expansion is continuing (Table 2) and larger flowers, wherein the petal tube of transgenic plants is on average longer than the petal tube from flowers on untransformed plants.

Also the number of flowers was increased in transgenic plants, as well as the number of fertilized flowers, resulting in a larger number of seed pods, and a greater seed yield per plant (data summarized in Table 4A). Moreover, the number of seeds per pod was larger in the transgenic plants than in the wild-type control plants. The aberrant seed yield in line C8-T1-6, was due to excessive high percentage of flower abscission.

It can thus be concluded that constitutive expression of AthCycD2 encoding DNA, leads to an increase both in number of seed pods and total yield of seeds on a per plant basis.

Finally, the root development in wild-type seedlings and transgenic seedlings was compared (Table 4B). Seeds were sterilized, sown on GM media plates without selection, vernalized and then stored in the vertical position in the growth room. Root length was measured 9 days and 13 days after vernalization and the presence of lateral roots recorded. Seeds from line C8-T1-7 and C8-T2-2 (homozygous) were used. Line C8-T1-7 possesses two inserts which segregate approximately 15 :1 on kanamycin plates. 35 seedlings were grown from this line and of these, three appeared to represent the rate of growth observed in wild type seedlings. Data from these seedlings was recorded separately nine days after vernalization. The t-test was applied to determine the significance of the mean difference and the level of significance is indicated in the table. ns denotes no significant difference between the samples. It thus seems that the increase in vegative growth in the apical parts is balanced by an equal increase in the root development.

Table 1. Mean height (in cm) of transformed tobacco plants comprising CaMV35S-AtcycD2

Line	Week 1 (45 days)	Week 2 (51 days)	Week 3 (59 days)	Week 4 (65 days)	Week 5 (73 days)	Week 6 (81 days)	Week 7 (89 days)
C8-T1-1	5.86 ± 2.45	13.71 ± 3.43	38.91 ± 6.61	63.09 ± 9.69	99.42 ± 10.25	123.30 ± 24.60	137.09 ± 31.90
C8-T1-2	8.50 ± 1.23	18.29 ± 2.21	49.86 ± 4.73	77.64 ± 4.73	117.14 ± 10.71	147.71 ± 17.75	168.00 ± 9.93
C8-T1-3	8.61 ± 2.59	17.41 ± 4.94	43.14 ± 9.08	66.61 ± 11.04	100.41 ± 15.57	134.95 ± 21.17	145.73 ± 22.15
C8-T1-5	6.81 ± 1.16	16.31 ± 1.89	44.38 ± 3.66	70.69 ± 5.30	106.50 ± 6.12	143.63 ± 12.33	159.88 ± 9.88
C8-T1-6	4.83 ± 1.75	10.75 ± 2.51	33.10 ± 6.47	52.67 ± 5.83	84.83 ± 8.08	120.50 ± 13.73	141.80 ± 5.63
C8-T1-7	8.64 ± 3.04	18.82 ± 4.56	48.32 ± 6.12	74.66 ± 7.12	111.50 ± 8.38	149.59 ± 11.05	169.14 ± 11.99
C8-T1-8	5.50 ± 1.41	13.2 ± 2.41	41.2 ± 2.17	62.40 ± 3.98	97.40 ± 8.08	134.20 ± 5.63	156.80 ± 11.86
C8-T1-9	3.75 ± 1.44	10.58 ± 3.32	35.67 ± 6.80	62.17 ± 9.72	100.67 ± 12.24	137.83 ± 20.34	162.17 ± 18.67
C8-T1-10	9.88 ± 1.89	21.00 ± 4.08	47.75 ± 8.02	75.38 ± 7.11	113.75 ± 9.21	152.75 ± 11.99	164.50 ± 177.21
C8-T1-11	10.00 ± 2.30	19.51 ± 3.82	45.17 ± 5.63	72.25 ± 5.50	103.33 ± 11.52	144.58 ± 5.63	152.83 ± 18.28
C8-T1-12	9.20 ± 2.66	17.9 ± 5.15	42.8 ± 11.01	68.6 ± 13.32	103.40 ± 14.40	140.80 ± 12.16	161.8 ± 10.76
wild-type	4.48 ± 1.63	10.50 ± 3.33	31.82 ± 6.62	54.00 ± 7.89	86.56 ± 10.91	121.81 ± 18.28	145.18 ± 19.44

Table 2. Mean leaf length (in cm) of the largest leaf of transformed tobacco plants comprising CaMV35S-AtcycD2

Line	Week 1	Week 2	Week 3
C8-T1-1	13.500 ± 1.846	19.909 ± 2.004	27.114 ± 2.182
C8-T1-2	16.643 ± 1.282	23.500 ± 1.354	29.643 ± 1.842
C8-T1-3	15.955 ± 2.400	20.951 ± 3.737	27.341 ± 3.095
C8-T1-5	15.062 ± 1.635	21.563 ± 1.741	28.875 ± 2.372
C8-T1-6	14.667 ± 1.402	20.833 ± 1.807	29.927 ± 1.201
C8-T1-7	15.886 ± 1.718	22.000 ± 1.498	28.909 ± 1.974
C8-T1-8	14.167 ± 2.229	20.500 ± 1.871	27.583 ± 1.856
C8-T1-9	12.417 ± 2.035	18.917 ± 2.010	26.333 ± 2.113
C8-T1-10	14.750 ± 2.693	20.167 ± 2.825	27.583 ± 2.635
C8-T1-11	15.833 ± 2.113	21.333 ± 1.602	28.333 ± 1.722
C8-T1-12	14.600 ± 1.432	21.400 ± 1.475	28.100 ± 2.608
wild-type	12.676 ± 1.846	18.691 ± 2.280	26.352 ± 1.960

Table 3A. Floral development [mean height to infloresence of 0.25cm or 1cm (in cm), mean time required to reach the development of an infloresence of 0.25 or 1cm (in days after vernalization)] in tobacco transformed with CaMV35SAthCycD2

Line	Mean time to inflorescence of 0.25cm (days)	Mean time to inflorescence of 1cm (days)	Mean height at infloresence of 1 cm (cm)
C8-T1-1	67.35 ± 4.580	74.75 ± 4.541	105.5 ± 21.670
C8-T1-2	65.42 ± 2.573	72.00 ± 3.546	110.6 ± 21.439
C8-T1-3	68.77 ± 3.436	74.32 ± 3.414	106.5 ± 14.134
C8-T1-5	70.25 ± 2.712	76.63 ± 2.387	122.4 ± 6.737
C8-T1-6	68.00 ± 2.828	73.33 ± 2.944	117.0 ± 5.550
C8-T1-7	70.95 ± 3.034	77.15 ± 2.852	133.4 ± 14.497
C8-T1-8	72.60 ± 3.286	77.60 ± 2.793	116.1 ± 5.482
C8-T1-9	73.17 ± 3.251	79.50 ± 2.429	129.25 ± 10.324
C8-T1-10	72.50 ± 2.517	77.75 ± 2.986	127.7 ± 19.202
C8-T1-11	66.67 ± 1.033	73.17 ± 2.137	104.6 ± 4.924
C8-T1-12	70.00 ± 4.000	76.40 ± 3.715	121.6 ± 7.893
mean value	69.61 ± 2.587	75.69 ± 2.329	117.70 ± 10.082
wild-type	74.90 ± 3.222	79.09 ± 2.342	111 ± 10.020

Table 3B. Floral development [mean flower size i.e., length and width (mm)] in tobacco transformed with CaMV35SAthCycD2.The length and width of five flowers from each plant was measured and the mean flower length or width for each transgenic line was calculated. The values for each independent transgenic line were compared to wild type using the t-test. The table reveals the level of probability that the results are statistically significant compared to wild type. ns means not significant.

Line	Mean flower length (mm)	Level of significance	Mean flower width (mm)	Level of significance
C8-T1-1	47.22 ± 2.261	P < 0.001	33.45 ± 1.668	P < 0.001
C8-T1-2	44.19 ± 1.848	P < 0.01	31.14 ± 1.486	P < 0.001
C8-T1-3	41.30 ± 1.720	ns	31.04 ± 1.360	P < 0.001
C8-T1-5	47.30 ± 2.822	P < 0.002	33.30 ± 1.945	P < 0.001
C8-T1-6	50.78 ± 1.990	P < 0.001	34.25 ± 1.467	P < 0.001
C8-T1-7	48.04 ± 2.604	P < 0.001	33.19 ± 1.391	P < 0.001
C8-T1-8	42.90 ± 1.252	ns	30.13 ± 2.270	ns
C8-T1-9	45.12 ± 1.906	P < 0.01	30.56 ± 1.333	P < 0.002
C8-T1-10	44.60 ± 1.627	P < 0.01	30.93 ± 2.002	P < 0.01
C8-T1-11	42.40 ± 1.891	ns	29.10 ± 2.998	ns
C8-T1-12	42.57 ± 0.978	P < 0.05	28.55 ± 1.190	P < 0.05
mean value	45.093 ± 2.877	P < 0.002	31.43 ± 1.886	P < 0.001
wild-type	41.22 ± 1.005	-	26.76 ± 1.099	

Table 4A. Mean number of seed pods per plant , mean weight of the seed content of six pods (g), mean seed yield per plant (g), in tobacco transformed with CaMV35SAthCycD2

Line	Mean number of seed pods	mean weight of seed content of six pods (g)	Mean seed yield per plant (g)
C8-T1-1	105.15 ± 14.96	1.085 ± 0.174	19.015
C8-T1-2	127.29 ± 7.82	0.824 ± 0.137	17.481
C8-T1-3	110.46 ± 16.30	1.106 ± 0.179	20.361
C8-T1-5	97.86 ± 10.81	1.105 ± 0.178	18.023
C8-T1-6	78.60 ± 12.97	1.078 ± 0.150	14.123
C8-T1-7	118.17 ± 15.64	1.131 ± 0.253	22.275
C8-T1-8	123.75 ± 4.78	1.123 ± 0.165	23.162
C8-T1-9	110.83 ± 20.91	1.090 ± 0.218	20.134
C8-T1-10	104.20 ± 10.99	1.122 ± 0.311	19.485
C8-T1-11	138.20 ± 8.35	1.116 ± 0.222	25.705
C8-T1-12	106.20 ± 12.62	1.134 ± 0.056	20.072
wild-type	106.73 ± 16.47	0.938 ± 0.118	16.685

Table 4B. Comparison of root development in wild-type and transgenic seedlings

Line	Number of plants	% Lateral roots	Mean root length (mm)		Level of significance
9 days after vernalization					
WT	23	36	15.326	± 1.893	
C8-T1-7	25	100	26.520	± 1.971	0.001
	3	33	14.000	± 3.464	ns
C8-T2-2	28	100	26.911	± 2.064	0.001
13 days after vernalization					
WT	16	100	28.188	± 1.893	
C8-T1-7	13	100	53.846	± 1.971	0.001
C8-T2-2	15	100	51.267	± 3.464	0.001

3.2. Tobacco plants comprising the CaMV35S-AthCycD3 chimeric gene.

Plants comprising the CaMV35S-AthCycD3 chimeric genes, were grown from T1 seeds and treated as described under 3.1. Measurements were started at 49 days after germination, with intervals of about 7 days. The following number of plant lines were analyzed for each line: 11 plants for line 1K9; 19 plants for line 3K9, 20 plants for line 4K9 and 18 plants for the untransformed control.

The following parameters were analyzed: the petal tube length and width (in cm) and the time (in days) at which at least 75% of the plants have reached at least the stage wherein an infloresence is clearly developed, summarized in Table 5.

Table 5. Summary of the measurements on tobacco plants comprising the CaMV35S-AthCycD3 chimeric gene.

Line	Mean petal tube length (cm)	mean petal tube width (cm)	mean time required to reach inflorescence of 1 cm (days)
1K9	5.66 ± 0.46	3.44 ± 0.27	100
3K9	5.18 ± 0.37	3.20 ± 0.35	100
4K9	5.48 ± 0.38	2.90 ± 0.35	93
wt	3.96 ± 0.12	2.39 ± 0.10	84

These transgenic plants had larger flowers, wherein the petal tube of transgenic plants was on average longer than the petal tube from flowers on untransformed plants, and also required more time to reach the stage wherein an inflorescence is clearly developed.

Example 4. Isolation of cycD-homologous genes from other plants

A c-DNA library, made from exponentially growing tobacco BY-2 cells was constructed in a Lambda Zap Express vector (Stratagene). Approximately 7.5 x 10^5 library clones were plated out, and replica blots made from each plate using Hybond N^+ nylon membranes (Amersham Int.) which were then fixed by baking at 80°C for two hours. The membranes were hybridized with *cyc*D2 or *cyc*D3 heterologous probes labelled with α-^{32}P dCTP by random priming. The *cyc*D3 probe comprised a *cyc*D3 fragment from *A. thaliana* (405 bp *Hinc*II-*Kpn*I fragment; having the nucleotide sequence of EMBL Accesion N° X83371 from nucleotide position 557 to nuceotide position 962). The *cyc*D2 probe consisted of an 1298 bp *Nco*I-*Sac*I fragment of *cyc*D2 from *A. thaliana* (having the nucleotide sequence of EMBL Accesion N° X83370 from nucleotide position 194 to nuceotide position 1332). *cyc*D3 hybridizations were carried out at 55°C and the membranes were washed for 10 min in 2xSSC/0.1% SDS twice, followed by a single 10 min wash in 0.1 SSC/0.1% SDS prior to autoradiography. The *cyc*D2 hybridizations were carried out at 48 °C; the membranes were washed for 10 min in 2x SSC/0.1% SDS three times. All washes were carried out at room temperature. Isolated library clones were excised *in vivo* (according to the manufacturer's protocol) to generate subclones in the pBK-CMV phagemid (Stratagene) and DNA sequence was determined according to standard methods. Sequence information was analyzed using the GCG (Genetics Computer Group) Software (1994). The sequences of *cyc*D2;1,

*cyc*D3;1and *cyc*D3;2 cDNAs from tobacco are represented in respectively, SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3.

Another cDNA library was made from polyadenylated RNA isolated from tubers, roots and leaves of *Helianthus tuberosus*. The cDNA was synthesized from an oligo (dT) primer and ligated into lambda ZAPII vector at the *Eco*RI site.

Approximately 1.25 x 10^6 clones were plated out, replica plaque blots were made as described above and hybridized using the labelled probes mentioned above. In addition the blots were screened with a *cyc*D1 probe, comprising the 401 bp *Xba*I-*Ava*I fragment of *cyc*D1 gene of *A. thaliana (*having the nucleotide sequence of EMBL Accesion N° X83369 from nucleotide position 312 to nuceotide position 713). Isolated clones were analyzed as above. The sequence of *cyc*D1;1 and *cyc*D3;1 genes from *Helianthus tuberosus* is represented in SEQ ID No. 4 and SEQ ID No. 5, respectively.

Yet another cDNA library was made from polyadenylated RNA isolated from callus material of *Zea mays* (Pa91xH99)xH99. The cDNA was synthesized from an oligo (dT) primer and ligated into lambda ZAPII vector at the *Eco*RI site. Approximately 1.25 x 10^6 clones were plated out, replica plaque blots were made as described above and hybridized using the labelled probes mentioned above. Isolated clones were analyzed as above. The sequence of the *cyc*D2 cDNA from *Zea mays* is represented in SEQ ID No. 21.

Example 5. Construction of the antisense chimeric genes and transformation of tobacco.

A 1298 bp *Nco*I-*Sac*I fragment comprising the DNA encoding CYCD2 from *A. thaliana* (having the nucleotide sequence of EMBL Accesion N° X83370 from nucleotide position 194 to nuceotide position 1332) was treated with Klenow polymerase to render the protruding termini blunt, and ligated to *Sma*I linearized pART7 (Gleave, 1992). A plasmid was selected wherein the inserted DNA fragment was in such an orientation that the DNA encoding the CYCD2 was introduced in the reverse way between a CaMV35S promoter of the CabbB-J1 isolate (Harpster *et al.*,1988) and a 3'*ocs* region (MacDonald *et al.*, 1991) , so that upon expression an antisense RNA is produced.

The chimeric antisense gene was then inserted between the T-DNA border of a T-DNA vector, comprising also a selectable chimeric marker gene. To this end, the chimeric *cyc*D2 gene was excised from pCEC2, using *Not*I, and ligated to *Not*I linearized pART27 (Gleave *et al.*, 1992) to create pCEC6.

Tobacco plants were transformed with this chimeric genes as described in Example 2.

Example 6. Analysis of the transformants

Plants transformed with the chimeric genes of Example 5 were treated as described in Example 3.1 and the following number of plants were analyzed: 7 plants for line C9-2, and 6 plants for line C9-7.

The following parameters were analyzed : height of the plants from the soil surface to the highest point (summarized in Table 6 as mean height ± standard deviation in cm); length of the largest leaf at defined times (summarized in Table 7 as mean length ± standard deviation in cm); time (summarized in Table 8 as mean time ± standard deviation in days) at which an infloresence merisitem is visible with the naked eye; height at which an infloresence meristem is visible (summarized in Table 8 as mean length ± standard deviation in cm) .

The transgenic plants exhibited a decreased growth rate, apparent from the seedling stage, resulting in a smaller average stem height (Table 6). The decreased growth rate also resulted on average in smaller leaves at the indicated times, which correspond to a period when leaf expansion is continuing (Table 7).

Table 6. Mean height (in cm) of transformed tobacco plants comprising CaMV35S-antisense *cyc*D2

Line	C9-2	C9-7	untransformed control
Week 1	2.64 ± 1.22	2.75 ± 0.89	4.48 ± 1.63
Week 2	6.64 ± 1.68	6.07 ± 1.43	10.50 ± 3.33
Week 3	20.00 ± 3.74	17.21 ± 6.47	31.82 ± 7.89
Week 4	34.07 ± 6.13	28.50 ± 5.83	54.00 ± 7.89
Week 5	54.00 ± 8.87	45.14 ± 8.46	86.56 ± 10.91
Week 6	74.29 ± 9.97	61.29 ± 5.11	121.80 ± 18.28
Week 7	85.92 ± 12.03	71.50 ± 23.19	145.18 ± 19.44

Table 7. Difference in mean leaf length of the largest leaf of transformed tobacco plants comprising CaMV35Santisense *cyc*D2 and the mean leaf length of the largest leaf of untransformed tobacco plants (in cm).

Line	C9-2	C9-7	untransformed control
Week 1	-2.31	-4.30	0
Week 2	-3.26	-6.44	0
Week 3	-4.35	-8.91	0

Table 8A. Mean flower size (mm), mean height to infloresence(cm), mean time required to reach the development of an infloresence (days) in tobacco transformed with CaMV35S antisense *cyc*D2.

Line	Mean time to infloresence (days)	Mean height to infloresence (cm)	Mean flower length (mm)
C9-2	102[a]	95	NA
C9-7	89 ± 7.95	68.57 ± 9.62	38.31
untransformed control	79 ± 2.39	111 ± 10.02	41.22

[a]Only one plant developed an infloresence during the monitoring period.

Table 8B. The effect of antisense CycD2 expression on flower length of transgenic tobacco was analyzed in other lines (T1 generation) and statistically compared to wild type using the student t-test . The length of five flowers from each plant was measured and the mean flower length for each transgenic line was calculated. The values for each independent transgenic line were compared to wild type using the t-test. The table reveals the level of probability that the results are statistically significant compared to wild type.

Line	Mean flower length (mm)	Level of significance
C9-T1-1	41.05 ± 1.558	ns
C9-T1-3	40.68 ± 1.574	ns
C9-T1-7	38.68 ± 1.991	ns
C9-T1-10	39.78 ± 1.024	$P < 0.05$
C9-T1-12	39.55 ± 1.568	$P < 0.05$
Mean value	40 ± 1.301	$P < 0.05$
wild type	41.22 ± 1.005	-

Example 7. Transformation of oil seed rape with the T-DNAs of Example 1 and similar vectors and analysis of transformed plants.

Hypocotyl explants of *Brassica napus* are obtained, cultured and transformed essentially as described by De Block *et al.* (1989), except for the following modifications:

- hypocotyl explants are precultured for 1 day on A2 medium [MS, 0.5 g/l Mes (pH5.7), 1.2% glucose, 0.5% agarose, 1 mg/l 2,4-D, 0.25 mg/l naphthalene acetic acid (NAA)and 1 mg/l 6-benzylaminopurine (BAP)].
- infection medium A3 is MS, 0.5 g/l Mes (pH5.7), 1.2% glucose, 0.1 mg/l NAA, 0.75 mg/l BAP and 0.01 mg/l gibberellinic acid (GA3).
- selection medium A5G is MS, 0.5 g/l Mes (pH5.7), 1.2% glucose, 40 mg/l adenine.SO_4, 0.5 g/l polyvinylpyrrolidone (PVP), 0.5% agarose, 0.1 mg/l NAA, 0.75 mg/l BAP, 0.01 mg/l GA3, 250 mg/l carbenicillin, 250 mg/l triacillin, 5 mg/l $AgNO_3$ for three weeks. After this period selection is continued on A5J medium (similar a A5G but with 3% sucrose)
- regeneration medium A6 is MS, 0.5 g/l Mes (pH5.7), 2% sucrose, 40 mg/l adenine.SO_4, 0.5 g/l PVP, 0.5% agarose, 0.0025mg/l BAP and 250 mg/l triacillin.
- healthy shoots are transferred to rooting medium which was A9: half concentrated MS, 1,5% sucrose (pH5.8), 100 mg/l triacillin, 0.6 % agar in 1 liter vessels.

MS stands for Murashige and Skoog medium (Murashige and Skoog, 1962).

Hypocotyl explants are infected with *Agrobacterium tumefaciens* strain C58C1Rif^R carrying a helper Ti-plasmid such as pGV4000 which is a derivative of pMP90 (Koncz and Schell, 1986) obtained by insertion of a bacterial chloramphenicol resistance gene linked to a 2.5 kb fragment having homology with the T-DNA vector pGSV5, into pMP90; and a T-DNA vector derived from pGSV5 comprising between the T-DNA borders the chimeric genes of Example 1 and the chimeric marker gene (pCEC5b and pCRK9b).

Transgenic oilseed rape plants comprising the chimeric genes of the invention, exhibit an accelerated vegetative program (increased growth rate), a reduction in the time required to reach the flowering stage, an increased number of flowers and an increased seed yield per plant.

Example 8. Transformation of corn plants with the vectors of Example 1 and similar vectors and analysis of the transformed plants.

Corn plants are transformed with the vectors of Example 1, according to WO92/09696. Transgenic corn plants comprising the chimeric genes of the invention exhibit an accelerated vegetative program (increased growth rate), a reduction in the time required to reach the flowering stage, an increased number of flowers and an increased seed yield per plant.

Example 9. Transformation of tomato plants with the vectors of Example 1 and similar vectors and analysis of the transformed plants.

Tomato plants are transformed with the vectors of Example 1, according to De Block *et al.* (1987) Transgenic tomato plants comprising the chimeric genes of the invention exhibit an accelerated vegetative program (increased growth rate), a reduction in the time required to reach the flowering stage, an increased number of flowers and an increased fruit yield per plant.

Example 10 Transformation of lettuce plants with the vectors of Example 1 and similar vectors and analysis of the transformed plants.

Lettuce plants are transformed with the vectors of Example 1, according to Micheimore *et al.* (1987). Transgenic lettuce plants comprising the chimeric genes of the invention exhibit an accelerated vegetative program (increased growth rate), a reduction in the time required to reach the flowering stage, an increased number of flowers and an increased seed yield per plant.

Example 11 Further phenotypic analysis of the progeny of the transgenic tobacco lines transformed with the CaMV35SAthCycD2 constructs of Example 3 in segregating and non-segregating populations.

Progeny populations (either segregating or non-segregating) of plants from two transgenic tobacco lines transformed with the CaMV35SAthCycD2 constructs (line 2 and line 5 of Example 3) were analyzed for length of time to flowering and increase in vegetative growth by measuring the mean heigth of the stem or the mean dry weight of the plants.

Segregation of the transgenes was monitored by establishing their resistance to kanamycine. For segregating populations, 32 plants were analyzed, while for non-segregating populations, 12 plants were analyzed. The non-transformed population consisted also of 12 plants.

The following populations were used:

Segregating populations:

Line 2

C8-T1-2 [T1 seed from C8-2 primary transformant; segregates 3:1 for T-DNA]

C8-T2-2 [T2 seed from C8-T1-2 plant #3 selfed, which was hemizygous and thus seed segregates 3:1 for T-DNA]

C8-T2-2 [T2 seed from a cross of C8-T1-2 plant #3 to wild type plant using wild type as pollen parent. This seed segregates 1:1 for T-DNA, and all T-DNA containing plants are hemizygous]

Line 5

C8-T1-5 [T1 seed from C8-5 primary transformant; segregates 3:1 for T-DNA]

C8-T2-5 [T2 seed from C8-T1-5 plant #304 selfed, which was hemizygous and thus seed segregates 3:1 for T-DNA]

Non-segregating populations

Line 2

C8-T2-2 [T2 seed from C8-T1-2 plant #302 selfed, which was homozygous for T-DNA]

Line 5

C8-T2-5 [T2 seed from C8-T1-5 plant #121 crossed to wild type plant using wild type as pollen parent. Plant #121 was homozygous for T-DNA and all T2 seed is hemizygous for the T-DNA]

C8-T2-5 [T2 seed from C8-T1-5 plant #121 selfed. Plant #121 was homozygous for T-DNA and all T2 seed is homozygous for the T-DNA].

The effect of CycD2 overexpression on the length of time to initiate inflorescence development in transgenic tobacco was measured and statistically compared to values for the same parameter measured for a wild type control population, using a non-parametric t-test in which the variances of the wt and transgenic populations were not assumed to be equal. The length of the time for each plant to develop an inflorescence of 0.5 cm was recorded and the mean number of days , post-vernalization was calculated. The values for each transgenic population was compared to the value for the wild-population using the t-test. The data for the segregating lines were separated in data for the kanamycin resistant population and the kanamycin sensitive population. The data for the kanamycin resistant population were also indicated separately for the homozygous kanamycin resistant subpopulation (not further segregating) and the hemizygous kanamycin resistant subpopulation (further segregating 3:1). In Table 9 these data are summarized. Table 10 summarizes the mean values of the stem heights in transgenic non-segregating lines at different timepoints post-vernilization, in comparison with a wild type population (statistically analyzed). A significance level of less than 0.05 is considered a highly significant difference between the mean height of each transgenic line and the mean height of the controls. ns indicates there is no significant difference between the populations. In addition, the biomass of seedlings from the mentioned non-segregating populations was compared to wild type seedlings during early vegetative growth . Seedlings were harvested at the days indicated after vernalisation and weighed before drying at 70 °C for 2 days. The mean dry weight of the seedlings and standard deviation was calculated and the results are presented in Table 11.

Table 9. The effect of CycD2 overexpression on the length of time to initiatiate inflorescence development in transgenic tobacco

Population	Mean time to inflorescence of 0.5 cm (days)	Standard deviation	Level of significance
Non-segregating lines			
WT	72.125	2.258	-
C8-T2-2 (302 selfed)	63.62	3.863	0.001
C8-T1-5 (121 selfed)	67.78	2.438	0.02
C8-T1-5 (121 x WT)	64.18	1.991	0.001
Mean value	65.19	2.258	0.001
Segregating lines			
C8-T1-2 selfed all Kan R	59.04	2.973	0.001
hemizygous	59.32	3.110	0.001
homozygous	58.50	2.507	0.001
Kanamycin sensitive	73.00	5.944	ns
C8-T2-2 pl 3 x WT			
Kanamycin resistant all	59.44	2.756	0.001
Kanamycin sensitive	69.10	2.846	ns
C8-T2-2 pl 3 selfed all Kan R	59.20	2.141	0.001
hemizygous	59.25	1.653	0.001
homozygous	59.38	3.021	0.001
Kanamycin sensitive	73.50	5.431	ns
C8-T1-5 All Kan R	62.67	3.367	0.001
Kanamycin sensitive	71.50	5.782	ns
C8-T2-5 pl 304 selfed			
hemizygous	64.50	3.030	0.001
homozygous	65.83	2.483	0.002
Kanamycin sensitive	74.50	7.764	ns

Table 10. Staticstical comparison of stem height of transgenic tobacco comprising CaMV35SAthCycD2 with wild type controls

Population	34 days	37 days	41 days	45 days	49 days	55 days	63 days	70 days	77 days	terminal height
wild type	1.48 ± 0.238	3.50 ± 0.831	7.59 ± 1.932	14.59 ± 3.816	25.81 ± 6.030	58.24 ± 8.423	107.06 ± 8.306	136.75 ± 7.105	153.94 ± 7.430	177.71 ± 13.129
C8-T2-2 (302 selfed)	3.55 ± 0.451	6.02 ± 1.662	11.11 ± 3.823	19.35 ± 6.528	32.81 ± 9.181	67.50 ± 12.281	120.12 ± 12.829	151.46 ± 15.253	165.23 ± 14.696	177.23 ± 13.935
level of significance	0.002	0.001	0.01	0.05	0.05	0.05	0.01	0.01	0.05	ns
C8-T2-5 (121 selfed)	4.25 ± 0.507	6.02 ± 1.662	11.11 ± 3.823	19.35 ± 6.528	32.81 ± 9.181	71.82 ± 7.604	119.86 ± 10.675	152.82 ± 11.297	170.73 ± 11.130	192.18 ± 7.846
level of significance	0.001	0.001	0.001	0.001	0.001	0.001	0.01	0.001	0.001	0.01
C8-T2-5 (121x WT)	4.37 ± 0.378	8.14 ± 1.914	14.76 ± 2.550	25.18 ± 3.314	39.91 ± 4.898	75.04 ± 6.258	117.83 ± 9.808	146.54 ± 13.422	160.71 ± 15.183	174.1 ± 14.963
level of significance	0.001	0.001	0.001	0.001	0.001	0.001	0.01	0.05	ns	ns

Table 11. Summary of dry weight measurements (in mg) obtained from non-segregating populations of transgenic seedlings overexpressing CycD2 and wild type (WT) seedlings at different time points post-vernilization. For all cases, the t-test indicates that there is a highly significant difference between the mean biomass of each transgenic line and the mean biomass of the controls.

Population	17 days	23 days	28 days	34 days	38 days
C8-T2-2 (302 selfed)	3.75 ± 1.462	22.11 ± 6.59	53.19 ± 9.97	337 ± 58.3	530 ± 60.0
C8-T2-5 (121 selfed	4.30 ± 1.623	29.05 ± 10.50	49.14 ± 8.51	351 ± 67.6	547 ± 24.9
C8-T2-5 (121x WT)	5.48 ± 1.130	39.51 ± 10.13	79.81 ± 20.36	476 ± 120.2	946 ± 154
Wild type	1.2 ± 0.510	13.16 ± 3.09	29.88 ± 14.89	135 ± 60.72	382 ± 90.3

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Cambridge University Technical Services Ltd.
(B) STREET: The Old Schools Trinity Lane
(C) CITY: Cambridge
(E) COUNTRY: United Kingdom
(F) POSTAL CODE (ZIP): CB2 1TS
(G) TELEPHONE: 44-1223334755
(H) TELEFAX: 44-1223332797

(ii) TITLE OF INVENTION: Plants with modified growth

(iii) NUMBER OF SEQUENCES: 21

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1284 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Nicotiana tabacum

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:182..1243
(D) OTHER INFORMATION:/note= "cDNA encoding cyclin CYCD2;1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CAAATTTTTC TCCCTTCTAT AGTCTCTTTC CTGTTCTCTT AAAAATCCTT AAAAATTTAT 60

TTTTTTTAAC AATCTCATGT AAATGGGATT AAATTTTGTA AAAATATAAG ATTTTGATAA 120

AGGGGGTTTA ATTATAACAT AGTAAATTAA GATTTTTTTT TTGCTTTGCT AGTTTGCTTT 180

AATGGCAGCT GATAACATTT ATGATTTTGT AGCCTCAAAT CTTTTATGTA CAGAAACAAA 240

AAGTCTTTGT TTTGATGATG TTGATTCTTT GACTATAAGT CAACAGAACA TTGAAACTAA 300

GAGTAAAGAC TTGAGCTTTA ACAATGGTAT TAGATCAGAG CCATTGATTG ATTTGCCAAG 360

TTTAAGTGAA GAATGCTTGA GTTTTATGGT GCAAAGGGAA ATGGAGTTTT TGCCTAAAGA 420

TGATTATGTC GAGAGATTGA GAAGTGGAGA TTTGGATTTG AGTGTGAGAA AAGAGGCTCT 480

TGATTGGATT TTGAAGGCTC ATATGCACTA TGGATTTGGA GAGCTGAGTT TTTGTTTGTC 540

GATAAATTAC TTGGATCGAT TTCTATCTCT GTATGAATTG CCAAGAAGTA AAACTTGGAC 600

AGTGCAATTG TTAGCTGTGG CCTGTCTATC ACTTGCAGCC AAAATGGAAG AAATTAATGT 660

TCCTTTGACT GTTGATTTAC AGGTAGGGGA TCCCAAATTT GTATTTGAAG GCAAAACTAT 720

ACAAAGAATG GAACTTTTGG TATTAAGCAC ATTGAAGTGG AGAATGCAAG CTTATACACC 780

TTACACATTC ATAGATTATT TTATGAGAAA GATGAATGGT GATCAAATCC CATCTCGGCC 840

GTTGATTTCT GGATCAATGC AACTGATATT AAGCATAATA AGAAGTATTG ATTTCTTGGA 900

ATTCAGGTCT TCTGAAATTG CAGCATCAGT GGCAATGTCT GTTTCAGGGG AAATACAAGC 960

AAAAGACATT GATAAGGCAA TGCCTTGCTT CTTCATACAC TTAGACAAGG GTAGAGTGCA 1020

GAAGTGTGTT GAACTGATTC AAGATTTGAC AACTGCTACT ATTACTACTG CTGCTGCTGC 1080

CTCATTAGTA CCTCAAAGTC CTATTGGAGT GTTGGAAGCA GCAGCATGCT TGAGCTACAA 1140

AAGTGGTGAT GAGAGAACAG TTGGATCATG TACAACTTCT TCACATACTA AAAGGAGAAA 1200

ACTTGACACA TCATCTTTAG AGCATGGGAC TTCAGAAAAG TTGTGAATCT GAATTTTCCC 1260

TTTTTAAAAA AAAAAAAAAA AAAA 1284

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1679 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Nicotiana tabacum

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:181..1299
(D) OTHER INFORMATION:/note= "cDNA encoding cyclin CYCD3;1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AAACGAGTCT CTGTGTACTC CTCCTCCTAT AGCTTTTCTC TCTTCTTCTC TTCACACCTC 60

CCACAACACA CAATCAGACA AAATAGAGAG GAAAATGAGT ATGGTGAAAA AGCTTTGTTT 120

TGTATAATGA GAAAAAGAGA TTTATATACA TCTCTTCTTC TACTTCCTTC TTACTAGAAG 180

ATGGCAATAG AACACAATGA GCAACAAGAA CTATCTCAAT CTTTTCTTTT AGATGCTCTT 240

TACTGTGAAG AAGAAGAAGA AAAATGGGGA GATTTAGTAG ATGATGAGAC TATTATTACA 300

CCACTCTCTT CAGAAGTAAC AACAACAACA ACAACAACAA CAAAGCCTAA TTCTTTATTA 360

CCTTTGCTTT TGTTGGAACA AGATTTATTT TGGGAAGATG AAGAGCTTCT TTCACTTTTC 420

TCTAAAGAAA AAGAAACCCA TTGTTGGTTT AACAGTTTTC AAGATGACTC TTTACTCTGT 480

TCTGCCCGTG TTGATTCTGT GGAATGGATT TTAAAAGTGA ATGGTTATTA TGGTTTCTCT 540

GCTTTGACTG CCGTTTTAGC CATAAATTAC TTTGACAGGT TTCTGACTAG TCTTCATTAT 600

CAGAAAGATA AACCTTGGAT GATTCAACTT GCTGCTGTTA CTTGTCTTTC TTTAGCTGCT 660

AAAGTTGAAG AAACTCAAGT TCCTCTTCTT TTAGATTTTC AAGTGGAGGA TGCTAAATAT 720

GTGTTTGAGG CAAAAACTAT TCAAAGAATG GAGCTTTTAG TGTTGTCTTC ACTAAAATGG 780

AGGATGAATC CAGTGACCCC ACTTTCATTT CTTGATCATA TTATAAGGAG GCTTGGGCTA 840

AGAAATAATA TTCACTGGGA ATTTCTTAGA AGATGTGAAA ATCTCCTCCT CTCTATTATG 900

GCTGATTGTA GATTCGTACG TTATATGCCG TCTGTATTGG CCACTGCAAT TATGCTTCAC 960

GTTATTCATC AAGTTGAGCC TTGTAATTCT GTTGACTACC AAAATCAACT TCTTGGGGTT 1020

CTCAAAATTA ACAAGGAGAA AGTGAATAAT TGCTTTGAAC TCATATCAGA AGTGTGTTCT 1080

AAGCCCATTT CACACAAACG CAAATATGAG AATCCTAGTC ATAGCCCAAG TGGTGTAATT 1140

GATCCAATTT ACAGTTCAGA AAGTTCAAAT GATTCATGGG ATTTGGAGTC AACATCTTCA 1200

TATTTTCCTG TTTTCAAGAA AAGCAGAGTA CAAGAACAGC AAATGAAATT GGCATCTTCA 1260

ATTAGCAGAG TTTTTGTGGA AGCTGTTGGT AGTCCTCATT AAAATCAATC ACCTGATTTA 1320

TCTCTTTTCT TTCTTATTAC CAACTATGGT GGTAATAATA TTTATTGATA TTCAGAAGTA 1380

TTTACCTTTA ATGTCATTTT CAAAAATTAC ATGAAAATGG AAAAAAAGAA AAGAAGAGCT 1440

TAGCTGGTGG TTGCAGTTGG CAGAGAAGAG GACTGGCTTT TTTTTGCAGG AGTGTAGTCT 1500

ACTACTACTG GAAAGCAGAG ATAGAGAGAG GAGAAAAGAC AGAAAATCTG CACTATTTGT 1560

TTTTTCTCTA TTCATATCAA TTCTCTCTTA GGTCCTTTTC ATGCATGCAT ACTTTTGATG 1620

GACATATTTT ATATATTTAC TATAATCATA AATTCTTGAA TAAAAAAAAA AAAAAAAAA 1679

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1431 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Nicotiana tabacum

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:198..1298
(D) OTHER INFORMATION:/note= "cDNA encoding cyclin CYCD3;2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CACCTTTACT CTCTTCTCCT TTTTGGCTCT TCCCATTCTC TCCTTCTCTT TCTTTATTTT 60

CTGTCCTGTA GAGAGAGAGA GAAAGTATAA GCAAAGCAGC AGATATGTTA CTGGGTCCAA 120

GATTGAGTTT TGGCTTACCT TGAAGATAAT GAGTAGAGCC TCCATTGTCT TCTTCCGTCA 180

AGAAGAAGAA GAAGAAGATG GTTTTCCCTT TAGATACTCA GCTCCTAAAT CCAATCTTTG 240

ATGTCCTTTA CTGTGAGGAA GATCGATTCT TGGACGATGA TGATTTAGGA GAATGGTCTA 300

GTACTTTAGA ACAAGTAGGA AATAATGTGA AAAAGACTCT ACCTTTATTA GAATGTGACA 360

TGTTTTGGGA AGATGACCAG CTTGTCACTC TTTTAACTAA GGAAAAAGAG TCTCATTTGG 420

GTTTTGATTG TTTAATCTCA GATGGAGATG GGTTTTTAGT GGAGGTTAGA AAAGAGGCAT 480

TGGATTGGAT GTTGAGAGTC ATTGCTCACT ATGGTTTCAC TGCTATGACT GCTGTTTTAG 540

CTGTGAATTA TTTTGATAGG TTTGTATCTG GACTCTGCTT TCAGAAAGAT AAGCCTTGGA 600

TGAGTCAACT TGCTGCTGTG GCTTGTCTTT CTATTGCTGC TAAAGTGGAA GAGACCCAAG 660

TCCCCCTTCT CTTAGACCTC CAAGTGGCTG ATTCAAGATT TGTGTTTGAG GCAAAGACTA 720

TTCAGAGAAT GGAACTCTTG GTGCTCTCCA CTCTTAAGTG GAAAATGAAT CCAGTGACAC 780

CACTATCTTT CATTGATCAT ATCATGAGGA GATTTGGATT CATGACCAAT CTACATTTGG 840

ATTTTCTTAG GAGATGTGAA CGCCTCATTC TTGGTATTAT CACTGATTCT AGGCTCTTGC 900

ATTATCCTCC ATCTGTTATT GCAACTGCAG TAGTGTATTT CGTGATCAAT GAGATTGAGC 960

CTTGCAATGC AATGGAATAC CAGAATCAGC TCATGACTGT TCTTAAAGTC AAACAGGATA 1020

GTTTTGAAGA ATGCCATGAT CTTATTCTAG AGCTAATGGG CACTTCTGGC TACAATATCT 1080

GCCAAAGCCT CAAGCGCAAA CATCAATCTG TACCTGGCAG TCCAAGTGGA GTTATCGATG 1140

CATATTTTAG TTGCGACAGC TCTAATGATT CGTGGTCGGT AGCATCTTCA ATTTCATCGT 1200

CACCAGAACC TCAGTATAAG AGGATCAAAA CTCAGGATCA GACAATGACA CTGGCTCCAC 1260

TGAGTTCTGT TTCTGTCGTT GTGGGCAGTA GTCCTCGTTG ATCAGTATCT CATTCTCTAG 1320

ATTATCTAGT ATTACGGCTA TGGTTACTAT ATGATCTCTC TTTTTTGGTA TGTTCTCTTA 1380

AACTGCAGTT GCACAATGCT CTGATGTTCC ATTAAAAAAA AAAAAAAAAA A 1431

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1788 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Helianthus tuberosus

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:165..1109
(D) OTHER INFORMATION:/note= "cDNA encoding cyclin CycD1;1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CACAACAATC ACTTCTACTC ACTATTCACT ACTTACTAAT CACTGCAACT TCTCCGGCCA 60

CTTTTCACCT CAAACCGCCG GAACTCCGCC GCTCCGGTCG ACGGTGAATC ACTGAATCTT 120

AGCAATTATG TTCACAACAG TATGAACAAT CAACACCGGT CATCATGTCA ATCTCGTGCT 180

CTGACTGCTT CTCCGACTTA CTCTGCTGCG AGGACTCCGG CATATTATCC GGCGACGACC 240

GGCCGGAGTG CTCCTATGAT TTCGAATATT CCGGCGACTT TGATGATTCG ATCGCGGAGT 300

TTATAGAACA GGAGAGAAAG TTCGTTCCAG GAATCGATTA CGTCGAGCGA TTTCAATCGC 360

AAGTTCTCGA TGCTTCTGCT AGAGAAGAAT CGGTTGCCTG GATCCTTAAG GTGCAACGGT 420

TTTACGGATT TCAGCCGTTG ACGGCGTACC TCTCCGTTAA CTATCTGGAT CGTTTCATCT 480

ATTGCCGTGG CTTCCCGGTG GCAAATGGGT GGCCCTTGCA ACTCTTATCT GTAGCATGCT 540

TGTCTTTAGC TGCTAAAATG GAGGAAACCC TTATTCCTTC TATTCTTGAT CTCCAGGTTG 600

AAGGTGCAAA ATATATTTTC GAGCCGAAAA CAATCCGAAG AATGGAGTTT CTTGTGCTTA 660

GTGTTTTGGA TTGGAGACTA AGATCCGTTA CACCGTTTAG CTTTATCGGC TTCTTTTCGC 720

ACAAAATCGA TCCATCTGGA ATGTATACGG GTTTCCTTAT CTCAAGGGCA ACACAAATTA 780

TCCTCTCAAA TATTCAAGAA GCTAGTTTAC TTGAGTATTG GCCATCATGT ATTGCTGCTG 840

CAACAATACT TTGTGCAGCA AGTGATCTTT CTAAATTCTC ACTTATCAAT GCTGATCATG 900

CTGAATCATG GTGTGATGGC CTTAGCAAAG AGAAGATCAC AAAATGTTAC AGACTTGTAC 960

AATCTCCAAA GATATTGCCG GTACATGTTC GAGTCATGAC GGCTCGAGTG AGTACTGAGT 1020

CAGGTGACTC ATCGTCGTCG TCTTCTTCGC CATCGCCTTA CAAAAAGAGG AAACTAAATA 1080

ACTACTCATG GATAGAGGAG GACAAAAGAT GAAAATAAGG AGACAAAATA AATAAATAAA 1140

TCCGGATTCC TCTCTATATT TTTTAAAGGA ATCAACAAAT ATATATAAAA AAAAAAAATG 1200

GAGTCAGGAA AAGCAACGAA AGCCGCCGGA GGAAGAAAAG GCGCCGGAGC GAGGAAGAAG 1260

TCCGTCACAA AGTCCGTCAA AGCCGGTCTC CAGTTCCCCG TCGGAAGAAT CGCTAGGTTT 1320

CTAAAAAAAG GCCGATACGC TCAACGTACC GGATCCGGAG CTCCGATCTA CCTTGCTGCT 1380

GTTCTAGAAT ACCTTGCTGC TGAGGTTTTG GAGTTGGCGG GAAATGCAGC GAGAGATAAC 1440

AAGAAGACAA GGATAAACCC TAGGCACTTG CTATTGGCTG TTAGGAACGA TGAGGAATTG 1500

GGGAAATTGC TTGCTGGTGT TACTATTGCT AGTGGAGGTG TGTTGCCCAA TATCAATCCG 1560

GTTCTTTTGC CCAAGAAGTC TTCTTCTTCT TCTGCTGCTG AGAAGACCCC CAAATCTAAA 1620

AAGTCGCCTA AAAAGGCTGC TTAGATAGAT GTTTCTGGTT ATAGTTGGTT AGATTAAGTT 1680

GAAGCAAAAC AGTCTCTTTT GTTCAATTAG TCGTCTGGCA ATGTAACTAT TTTGGTCGTC 1740

TTCAAAATGT TAATTGGATA CTATCTTCTT TAAAAAAAAA AAAAAAAA 1788

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1414 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Helianthus tuberosus

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:48..1118
(D) OTHER INFORMATION:/note= "cDNA encoding CYCD3;1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TTGAACCTTC ATTTCTTTTC TTTTCTTCTT TCTAATCACC AACCCCAATG GCCATTTTAT 60

CACCATATTC ATCTTCTTTC TTAGACACAC TCTTTTGCAA TGAACAACAA GATCATGAAT 120

ATCATGAATA TGAGTATGAA GATGAATTTA CACAAACCAC CCTCACAGAT TCATCTGATC 180

TCCATCTTCC CCCCCTGGAC CAACTAGATT TGTCATGGGA ACATGAAGAG CTTGTGTCCT 240

TGTTCACAAA AGAACAAGAG CAGCAAAAAC AAACCCCTTG TACTCTCTCT TTTGGCAAAA 300

CTAGTCCCTC AGTTTTTGCT GCTCGTAAAG AGGCTGTAGA TTGGATCCTT AAGGTCAAAA 360

GTTGTTATGG ATTCACACCT CTTACAGCCA TTTTAGCCAT CAATTATCTT GATAGGTTTC 420

TTTCTAGCCT CCATTTTCAA GAAGATAAAC CTTGGATGAT TCAACTTGTT GCTGTTAGTT 480

GTCTCTCTTT AGCTGCTAAA GTTGAAGAAA CTCAAGTGCC ACTCTTACTA GATCTTCAAG 540

TAGAGGACAC TAAGTACTTG TTTGAGGCTA AAAACATACA AAAAATGGAG CTTTTGGTGA 600

TGTCAACTTT GAAATGGAGG ATGAACCCAG TGACACCAAT CTCATTTCTT GATCACATTG 660

TAAGAAGGCT TGGATTAACT GATCATGTTC ATTGGGATTT TTTCAAGAAA TGTGAAGCTA 720

TGATCCTTTG TTTAGTTTCA GATTCAAGAT TCGTGTGTTA TAAACCATCC GTGTTGGCCA 780

CAGCTACAAT GCTTCACGTT GTAGATGAAA TTGATCCTCC CAATTGTATT GACTACAAAA 840

GTCAACTTCT GGATCTTCTC AAAACCACTA AGGACGACAT AAACGAGTGT TACGAGCTCA 900

TTGTCGAGCT AGCTTACGAT CATCACAACA AACGAAAACA TGATGCAAAC GAGACAACAA 960

CCAATCCGGT TAGTCCAGCT GGCGTGATCG ATTTCACTTG TGATGAAAGT TCAAATGAGT 1020

CATGGGAACT TAATGCTCAT CATTTCCGCG AGCCTTCATT CAAGAAAACA AGAATGGATT 1080

CAACAATTCG GGTTCGGGTT TGGTTCACTT ATAAGCTTTA ATCGAGGGTA GTTGTAAACA 1140

TGTAATCCGC ATGCACGCTA TTAATCCTAC GGTCCACTAC TACATATAAT CGGCCTATAA 1200

AATTATAGGT TAAGATGACC AGTCGTAGGC GTCGAGATGT CCTTATGGTT GGTCAATTTC 1260

TCTATGGTTT TAGGTCGTTT TTAATGTGAG ATAAATTAAA TTCGGTATGT TAAGTCTTTA 1320

TCAAGCAATG GACGTTATAT TTATTGTTTG ATATTGAGAA TTAAATTCCA TGGGAAAAAA 1380

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAA 1414

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA sequence of the T-DNA of PGSV5"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:1..25
(D) OTHER INFORMATION:/label= RB
/note= "right border sequence from the T-DNA of PGSV5"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:26..75
(D) OTHER INFORMATION:/label= MCS
/note= "Multiple Cloning Site"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:76..100
(D) OTHER INFORMATION:/label= LB
/note= "left border sequence from the T-DNA of pGSV5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

AATTACAACG GTATATATCC TGCCAGTACT CGGCCGTCGA CCGCGGTACC CGGGGAAGCT 60

TAGATCCATG GAGCCATTTA CAATTGAATA TATCCTGCCG 100

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 1 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GCMTGGATYC TYAAGGT 17

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 2 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TGCTTGTCWT TAGCTGC 17

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 3 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AAGAATGGAR YTTCTTGT 18

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 4 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

ARAGNATYCY KGCWGCAGC 19

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 5

for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CCRTCACACC AWGNYTCAG 19

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 6 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TGGWGATTTG GATTTG 16

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 7 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

ATNAANTACT TGGATCG 17

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 8 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

AGCTTGCANT CTCCANTTC 19

(2) INFORMATION FOR SEQ ID NO: 15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 9 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TCAGAAGNCC TGAANTC 17

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 10 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GANTGGATNY TNAARGT 17

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 11 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

AAGABAARCC WTGGATG 17

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 12 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GTKGAAGARA CTCAAGTBCC 20

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer 13 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TGGNGTNACW GGNTKCATYY TCCA 24

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer PR14 for PCR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GCWGNNGCNA NNNCAGANGG 20

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1846 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Zea mays

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:316..1389
(D) OTHER INFORMATION:/note= "cDNA encoding cyclin CYCD2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTGCAGTGGC CTAGCCGGCG TCGTCCTCCC CCTCTCHCGC TCCTCTGTCC TCCCCTCTCC 60

ACTTGAGAAG AACACAATTA GGAAAAAAAG GCAAAAAACA TTTACCTTTT TTCTATCTGT 120

ATATTATCTG AATAAATCAA GAGGAGGAAG AGGGGAGGGA GCGAGGGAGG GGGAGGAGTA 180

GCAAATCCAG ACTCCATAGC AACCAGCTCG CGAGAAGGGG AAAAGGGGGA GGAAGAGCTT 240

CGCTTGTGTA TTGATTGCTC GCTGCTCCAG TCCCTGCATT CGTGCCGTTT TTGGCAAGTA 300

GGTGGCGTGG CAAGCATGGT GCCGGGCTAT GACTGCGCCG CCTCCGTGCT GCTGTGCGCG 360

GAGGACAACG CTGCTATTCT CGGCCTGGAC GACGATGGGG AGGAGTCCTC CTGGGCGGCC 420

GCCGCTACGC CGCCACGTGA CACCGTCGCC GCCGCCGCCG CCACCGGGGT CGCCGTCGAT 480

GGGATTTTGA CGGAGTTCCC CTTGCTCTCG GATGACTGCG TTGCGACGCT CGTGGAGAAG 540

GAGGTGGAGC ACATGCCCGC GGAGGGGTAC CTCCAGAAGC TGCAGCGACG GCATGGGGAC 600

CTGGATTTGG CCGCCGTCAG GAAGGACGCC ATCGATTGGA TTTGGAAGGT CATTGAGCAT 660

TACAATTTCG CACCGTTGAC TGCCGTTTTG TCTGTGAACT ACCTCGATAG ATTCCTCTCC 720

ACGTATGAGT TCCCTGAAGG CAGAGCTTGG ATGACTCAGC TCTTGGCAGT GGCTTGCTTG 780

TCTTTGGCTT CGAAAATCGA AGAGACTTTT GTGCCACTCC CCTTGGATTT GCAGGTAGCG 840

GAGGCAAAGT TTGTTTTTGA GGGAAGGACC ATAAAAAGGA TGGAGCTTCT GGTGCTAAGC 900

ACCTTAAAGT GGAGGATGCA TGCTGTTACT GCTTGCTCAT TTGTTGAATA CTTTCTTCAT 960

AAATTGAGTG ATCATGGTGC ACCCTCCTTG CTTGCACGCT CTCGCTCTTC GGACCTTGTC 1020

TTGAGCACCG CTAAAGGTGC TGAATTCGTG GTATTCAGAC CCTCCGAGAT TGCTGCCAGT 1080

GTTGCACTTG CTGCTATCGG CGAATGCAGG AGTTCTGTAA TTGAGAGAGC TGCTAGTAGC 1140

TGCAAATATT TGGACAAGGA GAGGGTTTTA AGATGCCATG AAATGATTCA AGAGAAGATT 1200

ACTGCGGGAA GCATTGTCCT AAAGTCTGCT GGATCATCAA TCTCCTCTGT GCCACAAAGC 1260

CCAATAGGTG TCCTGGACGC TGCAGCCTGT CTGAGTCAAC AAAGCGATGA CGCTACTGTC 1320

GGGTCTCCTG CAGTATGTTA CCATAGTTCT TCCACAAGCA AGAGGAGAAG GATCACTAGA 1380

CGTCTACTCT AATTGTGGTA CGCTTCAGGT GTGCTCCTCA CCGCTCTAGG AGTTTTTGAT 1440

TGGTTCAAAC ATCTTAAATT TAGTTTGGCC GCTGGAGGAT TATGGTTTAG TCAAGTAGTT 1500

GCTGAATGGA CAACAAAACA CGCACACTAC TTGGTCCATA AAGACAAGAA AATAACTGGC 1560

AGCGTCCCGC GAGCCAGCGC TGCAATCCAG TTCATGCAAG ACCCTAGAGT CCAGGGGGGG 1620

TGCTGGTGTA GGTAGAGAGG GAACAAGGCA TTCACATACG CCGTAGAGAT GAGAGAGCCT 1680

CTCGTATGTT TTGTACTTTT GCTCCTTCAG TTTGCAATGA ACTATATAAA CAAGGATTGC 1740

CTTGGGGCAG TGAACATTTG TCGGATGAAA AGAATCAAAA AGGATGGGGG TCGGCAGAGG 1800

AATAGAACAA TTTGATATAT TTCCATAAAC TAAAAAAAAA AAAAAA 1846

Claims

1. A process to obtain a plant with altered growth characteristics or altered architecture, said process comprising the step of altering the level or functional level of a cell-division controlling protein within cells of a plant, wherein said cell-division controlling protein is capable of binding or phosphorylating an Rb-like protein.

2. The process of claim 1, wherein said cell-division controlling protein comprises an Rb-like protein binding motif in the N-terminal part of the protein.

3. The process of claim 2, wherein said Rb-like protein binding motif is LxCxE.

4. The process of claim 3, wherein said cell-division controlling protein is a D-type cyclin.

5. The process of any one of claims 1 to 4, wherein said level or functional level of said cell-division controlling protein is altered by expressing in said cells of said plant, a chimeric gene comprising the following operably linked DNA fragments:
a) a plant expressible promoter region
b) a transcribed DNA region encoding an RNA or a protein, which when expressed either increases or decreases said level or functional level of said cell-division controlling protein; and optionally
c) a 3' end formation and polyadenylation signal functional in plant cells.

6. The process of claim 5, wherein said transcribed DNA region encodes an antisense RNA, a ribozyme or a sense RNA strand, which when expressed reduces, inhibits or prevents the expression of said cell-division controlling protein.

7. The process of claim 6, wherein said transcribed DNA region encodes an antisense RNA, which when expressed reduces, inhibits or prevents the expression of an endogenous D-type cyclin.

8. The process of claim 5, wherein said transcribed DNA region encodes a cell-division controlling protein capable of binding or phosphorylating an Rb-like protein.

9. The process of claim 5, wherein said transcribed DNA region encodes a cell-division controlling protein comprising an Rb-like protein binding motif.

10. The process of claim 9, wherein said binding motif is LxCxE.

11. The process of claim 5, wherein said transcribed DNA region encodes a D-type cyclin.

12. The process of claim 11, wherein said D-type cyclin is a D-type cyclin from plants.

13. The process of claim 12, wherein said D-type cyclin is selected from *Arabidopsis thaliana* CYCD1, *Arabidopsis thalina* CYCD2, *Arabisopsis thaliana* CYCD3, *Nicotiana tabacum* CYCD3;1, *Nicotiana tabacum* CYCD2;1, *Nicotiana tabacum* CYCD3;2 , *Helianthus tuberosus* CYCD1;1 *Zea mays* CYCD2 and *Helianthus tuberosus* CYCD3.

14. The process of claim 12, wherein said transcribed DNA region comprises a nucleotide sequence selected from the nucleotide sequence of EMBL Accession N° X83369 from the nucleotide position 104 to the nucleotide position 1108, the nucleotide sequence of EMBL Accession N° X83370 from the nucleotide position 195 to the nucleotide position 1346, the nucleotide sequence of EMBL Accession N° X83371 from the nucleotide position 266 to the nucleotide position 1396, the nucleotide sequence of SEQ ID N° 1 from nucleotide position 182 to nucleotide position 1243, the nucleotide sequence of SEQ ID N° 2 from nucleotide position 181 to nucleotide position 1299, the nucleotide sequence of SEQ ID N° 3 from nucleotide position 198 to nucleotide position 1298, the nucleotide sequence of SEQ ID N° 4 from nucleotide position 165 to nucleotide position 1109, the nucleotide sequence of SEQ ID N° 5 from nucleotide position 48 to nucleotide position 1118 or the nucleotide sequence of SEQ ID N° 21 from nucleotide position 316 to nucleotide position 1389.

15. The process of claim 5, wherein said transcribed DNA region encodes a protein or peptide which when expressed increases said functional level of said cell division controlling protein.

16. The process of claim 15, wherein said transcribed DNA region encodes a protein or peptide selected from: a mutant D-type cyclin, a part of a D-type cyclin, a D-type cyclin which has a mutation in the cyclin box, a D2-type cyclin which has a substitution of amino acid 185 or amino acid 155, a D2-type cyclin which has mutation E185A or K155A, a D-type cyclin wherein the PEST sequences are removed, a D-type cyclin wherein the LxCxE binding motif has been changed or deleted, or a D-type cyclin wherein the C-residue from the LxCxE binding motif has been deleted.

17. The process of any one of claims 5 to 16, wherein said plant expressible promoter region is a CaMV35S promoter region.

18. The process of claim 6 or claim 7, wherein said altered growth characteristic comprises a reduced growth rate.

19. The process of any one of claims 8 to 17, wherein said altered growth characteristic comprises an increased growth rate.

20. The process of any one of claims 8 to 17, wherein said altered growth characteristic comprises a faster germination.

21. The process of any one of claims 8 to 17, wherein said altered growth characteristic comprises a reduction in time required to flower.

22. The process of any one of claims 8 to 17, wherein said altered architecture comprises an increased number of flowers per plant or an increased number of seeds per plant or an increased number of fruit per plant.

23. A chimeric gene as described in any one of claims 5 to 17.

24. A plant cell, comprising the chimeric genes of claim 23.

25. A plant, consisting essentially of the plant cells of claim 24.

26. The plant of claim 25, which is a greenhouse-grown plant.

27. The plant of claim 23, which plant is selected from pine, poplar, *Eucalyptus* tree, alfalfa, legumes, grasses, corn, oil seed rape, linseed, wheat, a brassica vegetable, tomato, lettuce, rice, barley, potato, tobacco, sugar beet, sunflower, carnation, chrysanthemum, rose, or tulip.

28. A seed of the plant of any one of claims 25 to 27, said seed comprising the chimeric genes of claim 23.

29. An isolated DNA fragment comprising the nucleotide sequence from SEQ ID N° 1 from the nucleotide at position 182 to the nucleotide at position 1243.

30. An isolated DNA fragment comprising the nucleotide sequence from SEQ ID N° 2 from the nucleotide at position 181 to the nucleotide at position 1299.

31. An isolated DNA fragment comprising the nucleotide sequence from SEQ ID N° 3 from the nucleotide at position 198 to the nucleotide at position 1298.

32. An isolated DNA fragment comprising the nucleotide sequence from SEQ ID N° 4 from the nucleotide at position 165 to the nucleotide at position 1109.

33. An isolated DNA fragment comprising the nucleotide sequence from SEQ ID N° 5 from the nucleotide at position 48 to the nucleotide at position 1118.

34. An isolated DNA fragment comprising the nucleotide sequence from SEQ ID N° 21 from the nucleotide at position 316 to the nucleotide at position 1389.

35. The use of a cell-division controlling protein, capable of binding the pocket domain of an Rb-like protein or capable of phosphorylating an Rb-like protein, to alter the growth characteristics or architecture of a plant.

36. The use of claim 35, wherein said cell-division controlling protein comprises an LxCxE binding motif in the N-terminal part of the protein.

37. The use of claim 36, wherein said cell-division controlling protein is a D-type cyclin.

38. The use of claim 37, wherein said cell-division controlling protein is a D-type cyclin selected from *Arabidopsis thaliana* CYCD1, *Arabidopsis thalina* CYCD2, *Arabisopsis thaliana* CYCD3, *Nicotiana tabacum* CYCD3;1, *Nicotiana tabacum* CYCD2;1, *Nicotiana tabacum* CYCD3;2, *Helianthus tuberosus* CYCD1;1, *Zea mays* CYCD2 and *Helianthus tuberosus* CYCD3.

39. The use of any one of claims 34 to 37, wherein said cell-division controlling protein is encoded by a chimeric gene, integrated in the genome of the cells of a plant.

40. The use of a DNA encoding a cell-division controlling protein capable of binding or phosphorylating an Rb-like protein to alter the growth characteristics or architecture of a plant.

41. The use of claim 40, wherein said cell-division controlling protein is a D-type cyclin.

42. The use of claim 41, wherein said DNA comprises a nucleotide sequence selected from the nucleotide sequence of EMBL Accession N° X83369 from the nucleotide position 104 to the nucleotide position 1108, the nucleotide sequence of EMBL Accession N° X83370 from the nucleotide position 195 to the nucleotide position 1346, the nucleotide sequence of EMBL Accession N° X83371 from the nucleotide position 266 to the nucleotide position 1396, the nucleotide sequence of SEQ ID N° 1

from nucleotide position 182 to nucleotide position 1243, the nucleotide sequence of SEQ ID N° 2 from nucleotide position 181 to nucleotide position 1299, the nucleotide sequence of SEQ ID N° 3 from nucleotide position 198 to nucleotide position 1298, the nucleotide sequence of SEQ ID N° 4 from nucleotide position 165 to nucleotide position 1109, the nucleotide sequence of SEQ ID N° 5 from nucleotide position 48 to nucleotide position 1118 or the nucleotide sequence of SEQ ID N° 21 from nucleotide position 316 to nucleotide position 1389.

INTERNATIONAL SEARCH REPORT

national Application No
PCT/EP 98/01701

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N15/29 A01H5/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 03631 A (SALK INST FOR BIOLOGICAL STUDI) 29 January 1998 see claim 1	1
Y	DOERNER, P., ET AL.: "Control of root growth and development by cyclin expression" NATURE, vol. 380, 11 April 1996, pages 520-523, XP002045509 cited in the application see the whole document	1-5, 8-17, 23-28, 35-42

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
27 August 1998	09/09/1998

Name and mailing address of the ISA	Authorized officer
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Y	SONI, R., ET AL.: "A family of cyclin D homologs from plants differentially controlled by growth regulators and containing the conserved retinoblastoma protein interaction motif" THE PLANT CELL, vol. 7, January 1995, pages 85-103, XP002045510 cited in the application see the whole document	1-5, 8-17, 23-28, 35-42
A	DOONAN, J.: "Plant growth: roots in the cell cycle" CURRENT BIOLOGY, vol. 6, no. 7, July 1996, pages 788-789, XP002045511 see the whole document	1-42
A	XIE, Q., ET AL.: "Plant cells contain a novel member of the retinoblastoma family of growth regulatory proteins" THE EMBO JOURNAL, vol. 15, no. 18, 1996, pages 4900-4908, XP002045512 cited in the application see the whole document	1-42
A	GRAFI, G., ET AL.: "A maize cDNA encoding a member of the retinoblastoma protein family: involvement in endoreduplication" PROC. NATL. ACAD. SCI.(USA), vol. 93, August 1996, pages 8962-8967, XP002042542 see the whole document	1-42
A	DAHL, M., ET AL.: "The D-type alfalfa cyclin gene cycMs4 complements G1 cyclin-deficient yeast and is induced in the G1 phase of the cell cycle" THE PLANT CELL, vol. 7, November 1995, pages 1847-1857, XP002045513 cited in the application see the whole document	1-42
A	HEMERLY, A., ET AL.: "Dominant negative mutants of the Cdc2 kinase uncouple cell division from iterative plant development" EMBO JOURNAL, vol. 14, no. 16, 1995, pages 3925-3936, XP002045514 cited in the application see the whole document	1-42

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/01701

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BELL, M.H., ET AL.: "Tobacco plants transformed with cdc25, a mitotic inducer from fission yeast" PLANT MOLECULAR BIOLOGY, vol. 23, 1993, pages 445-451, XP002045515 see the whole document	1-42
A	WO 92 09685 A (UNIV AUSTRALIAN) 11 June 1992 cited in the application see the whole document	1-42
A	RENAUDIN, J.-P., ET AL.: "Plant cyclins: a unified nomenclature for plant A-,B- and D-type cyclins based on sequence organization" PLANT MOLECULAR BIOLOGY, vol. 32, 1996, pages 1003-1018, XP002046019 see the whole document	1-42
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 98/01701

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(74) Agents: GUTMANN, Ernest et al.; Ernest Gutmann – Yves Plasseraud S.A., 3, rue Chauveau–Lagarde, F–75008 Paris (FR).

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(54) Title: PLANTS WITH MODIFIED GROWTH

(57) Abstract

A process is provided for modifying growth or architecture of plants by altering the level or the functional level of a cell division controlling protein, preferably a cell–division controlling protein that binds or phosphorylates retinoblasoma–like proteins, more preferably a cyclin, particularly a D–type cyclin within cells of a plant. Also provided are chimeric genes comprising a transcribed DNA region encoding an RNA or a protein, which when expressed either increases or decreases the level or functional level of a cell–division controlling protein, and plant cells and plants expressing such chimeric genes.

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